SOIL MICROBIAL COMMUNITY STRUCTURE AND FUNCTION ALONG ENVIRONMENTAL GRADIENTS: IMPLICATIONS FOR WETLAND NITROGEN CYCLING

BY

ARIANE LEGASPI PERALTA

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

Doctoral Committee:

Associate Professor Michelle M. Wander, Chair
Associate Professor Angela D. Kent, Director of Research
Associate Professor James W. Dalling
Adjunct Associate Professor Yong Cao

ABSTRACT

Riparian wetlands are sites of intense biogeochemical activity and play an important role in improving water quality and recycling nutrients through microbially-mediated ecosystem functions. To enhance restoration of nutrient cycling functions, it is critical to know how abiotic factors influence microbial community structure and ecosystem function. Microbial systems are especially important for investigating structure-function relationships because microbial taxa can be linked to specific biogeochemical transformations.

The goal of this study was to document factors that significantly contribute to plant and microbial community variation in order to identify potential environmental filters responsible for structuring communities among and within wetlands. Vegetation surveys and microbial community composition (assessed by molecular analysis) along with landscape and local soil factors were assessed at 27 restored wetlands. Surrounding land use and soil fertility, specifically inorganic nitrogen concentration, were associated with plant community variation. In contrast, local soil factors, especially soil pH, were strongly linked to bacterial and denitrifier community variation. Identifying environmental drivers that affect plant and microbial communities can inform managers of possible ecosystem functions that can be supported at a restoration site.

To better understand structure-function relationships, I focused on microbial functional guilds and activities for the processes of denitrification ($NO_3^- \rightarrow N_2$) and nitrification ($NH_4^+ \rightarrow NO_3^-$). Together, these nitrogen transformations influence nitrate removal capacity within wetlands. Denitrification is a facultatively anaerobic process, while microbes responsible for nitrification are obligate aerobes. Thus, these nitrogen transformations are particularly sensitive to oxygen concentration and soil moisture. Denitrifier and ammonia oxidizer composition were initially studied along an environmental gradient within a single wetland. I demonstrated that

denitrifier taxa occupied a wider moisture range compared to ammonia oxidizer taxa. To further investigate how environmental gradients related to microbial structure-function relationships, I compared denitrifier and ammonia oxidizer community composition and activity along an environmental gradient within different wetland sites. Denitrifier and ammonia oxidizer community composition were distinct between upland and wetland plots at all sites. Microbial community structure was relatively constant, whereas potential microbial activity decreased over time at most sites. In addition, potential denitrification and nitrification rates were mainly influenced by environmental conditions compared to community structure (community composition or abundance).

To understand if hydrologic history constrains contemporary microbial function, I investigated the response of initial microbial communities shaped by historical hydrologic regime to a drying/flooding treatment in a mesocosm experiment. Initial hydrologic history strongly affected community structure and function. Potential denitrification rate significantly increased under wetter conditions, whereas potential nitrification rates remained unchanged in many cases. Results suggest that denitrification activity was more sensitive to drying/flooding, whereas nitrification was constrained by the resident community structure. Variation in microbial response can result in a shift in dominant nitrogen cycling transformations within wetlands.

Restoring nitrate removal function by encouraging denitrification is not as straightforward as flooding an area. Evidence from this study suggests that nitrification, resulting in nitrate production, can still occur under saturated conditions (presumably low [O_2]) if not limited by low pH or ammonium. As a consequence, while trying to restore one microbial function, restoration practitioners must consider how other, potentially opposing biogeochemical functions will respond.

ACKNOWLEDGEMENTS

This dissertation is dedicated to the memory of my mother, Iris Legaspi Peralta. Her amazing character, work ethic, and strength have been a constant source of inspiration. I begin with personally thanking my advisor, Angela Kent for continued support and guidance during graduate school. I valued all the opportunities she gave me to learn, to be challenged, and to persevere. I would also like to thank the other members of my graduate committee – Yong Cao, Jim Dalling, and Michelle Wander for continued support, constructive feedback, and encouragement. I also thank Jeff Matthews for going above and beyond the duties of a collaborator. Our conversations about current and future projects have made me a better scientist. Thanks for being a great sounding board throughout the years. I also appreciate the statistical assistance from Rick Lankau, Tony Yannarell, Jeff Matthews, and Yong Cao. Members of my thesis committee, Jeff Matthews, Daniel Keymer, Emily Wheeler, Sara Paver, Greg Sypreas, and Chris Balakrishnan provided helpful comments on this dissertation. Thanks are also extended to Carol Augspurger and Jim Dalling for the opportunity to participate in an amazing teaching environment. You were truly inspirational educators and were always willing to help me improve as an instructor.

This work would not have been possible without the help of many in the lab and in field at the University of Illinois and Michigan State University. A big thanks goes to Daniel Keymer for always being the voice of reason and being the most helpful and constructive critic in the lab. I also want to thank Sara Paver, my ‘go to’ person for just about everything from manuscript edits to food runs. I also greatly appreciate the help of Diana Flanagan whose hard work greatly contributed to the wetlands survey project. Thanks are also extended to Robert Lane, Yu-rui Chang, Jason Koval, Ginny Li, Derrick Lin, Yi Lou, and Elizabeth Hu for generous feedback and

help in the field and lab. To Eric Johnston and Sarah Ludmer, thank you for being such hard working and bright students for whom I had the great opportunity to mentor. Thanks to Lauren, Elizabeth, Omar, Neil, and Jen for help in the lab and field. I am indebted to Candice Smith, Karen Starks, Corey Mitchell, Jenwei Tsai, and Krishna Woli of the David Lab for generously helping me with all things biogeochemical. I also thank Robert Darmody for the fruitful conversations about soil and for welcoming me into his lab. I would like to thank the students and professors at MSU's Kellogg Biological Station, especially Lauren Kinsman, Jason Martina, Jon O'Brien, Steve Hamilton, and Jay Lennon for support during my field season at KBS.

Thanks go out to Sara Paver, Emily Wheeler, Cassandra Allsup, and Katie Amato, for providing constructive feedback, support and generally great memories both of science and non-science related matters. Thanks are extended to my friends Johanna Salzer, Giusi Amore, Chris Balakrishnan, Jessica Girard, Greg Spyreas, Jason Fischer, John Drake, Carmen Ugarte, and Sarah London. I could always count on you for support both in and out of the science bubble. Thanks also to members of GEEB for fostering a positive grad school culture at UIUC.

Last but definitely not least, I thank my support network away from grad school. I would like to thank my father Marshall Peralta, uncle Rafaelito Legaspi, and aunt Consuelo Legaspi O'Toole for continued encouragement. Thanks for always supporting me and never questioning my life decisions even when they were confusing at times. I would also like to thank my family and extended families – the Legaspi's, Peralta's, Kerekes', Carlier's, Foster's, Leitz's, and Salzer's – I could not have had a bigger cheering section. To my extended Munster and Urbana families, thank you for keeping me from working too much and for reminding me of my former life as a musician. And finally, thank you to my Corkscrew and Blue Moon Farm families, you provided the balance I needed at the end of a long grad school road. The timing was impeccable.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

BACKGROUND

Implications of wetland loss

Anthropogenic modification of land and water use has led to increased erosion, sediment loads, and nutrient pollution in major waterways (David and Gentry 2000, Panno et al. 2008, Schneider 1996). Changing patterns of land use over the last century have resulted in the loss of about 60% of wetlands in the Midwestern United States (Zedler 2003). The loss of ecosystem services through conversion of wetlands to agriculture exacerbates nutrient run-off that contributes to eutrophication of downstream waters (Rabalais et al. 2002). Ultimately, inorganic nutrients originating from the agricultural application of nitrogen fertilizers are discharged to the Gulf of Mexico. These nutrients increase biotic activity and oxygen demand, resulting in the annual recurrence of a hypoxic zone off the Louisiana coast each summer during summer stratification (Rabalais et al. 2002). The deleterious effects and the increasing severity of hypoxic zones in the Gulf of Mexico and other coastal areas have led to increased interest in understanding and restoring ecosystem processes that can mitigate nitrate pollution.

Microbial function in wetlands

Riparian wetlands are sites of intense biogeochemical activity and play an important role in improving water quality, recycling nutrients, and detoxifying chemicals (Keddy 2000). Microbial processes play a key role in biogeochemical transformations essential for improving water quality by controlling virtually all nitrogen transformations in these systems (Fig. 1.1). Disturbance and altered hydrology associated with land use change may directly impact

microbial communities and their interactions with other organisms altering the structure of the microbial communities themselves (Duncan and Groffman 1994, Hooper et al. 2000, Hunter and Faulkner 2001, Schimel and Gulledge 1998, Wardle et al. 1998, Whitham et al. 2003). The link between microbial community structure and function in a restoration context is not well understood (Gutknecht et al. 2006). The inability to connect microbial community composition to important ecosystem processes hinders the development of predictive models for biogeochemical transformations, particularly in response to land use change and restoration management (Reed and Martiny 2007, Wallenstein et al. 2006). Restoring microbially-mediated wetland ecosystem functions will require a better understanding of how environmental factors influence microbial community composition and activity.

Defining function more clearly is an achievable goal for particular microbially-mediated ecosystem services such as nutrient cycling and water quality improvement. Specifically, a better understanding of the role of microbial communities in denitrification (anaerobic biological transformation of nitrate to nitrogen gas) and nitrification (aerobic transformation of ammonium to nitrate) would enhance wetland functional assessment methods (Stokstad 2008). Molecular microbial ecology methods allow us to examine the bacterial community and offer insight into the ecological drivers structuring microbial communities and influencing microbial activity (Hartman et al. 2008, Peralta et al. 2010). Molecular methods also allow us to focus on microbial populations responsible for specific ecosystem-level processes. Examination of the effect of soil characteristics as environmental filters on the community structure of microbial populations involved in specific biogeochemical transformations can inform us of the controls on the ecosystem process of interest (Luton et al. 2002, Rich et al. 2003, Wallenstein et al. 2006, Zak et al. 2006).

Processes and players in wetland nitrogen cycling

In order to understand the relationship between community structure and ecosystem functions, a detailed assessment of how microorganisms involved in nitrogen (N) cycling transformations respond to environmental gradients is needed. The majority of N cycling transformations are microbially-mediated and well-characterized (Francis et al. 2007, Wallenstein et al. 2006). Nitrate (NO_3^-) removal functions of ecosystems occur via the denitrification pathway ($NO_3^- \rightarrow N_2$), while internal N cycling occurs through nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$). Both of these transformations are particularly sensitive to oxygen concentrations, which are controlled by soil moisture. Another key factor in determining association between microbial community and structure is oxygen availability (Francis et al. 2007, Kowalchuk and Stephen 2001, Wallenstein et al. 2006). Denitrifiers are facultative anaerobes, meaning they carry out anaerobic respiration under anoxic conditions but will carry out aerobic respiration when oxygen is available. Denitrifiers live and function in a variety of environmental conditions and are a phylogenetically heterogeneous group (Goddard and Bradford 2003, Nogales et al. 2002, Rich and Myrold 2004, Wallenstein et al. 2006). Ammonia oxidizers, in contrast, are obligate aerobes and are restricted to few phyla within *Betaproteobacteria* and *Crenarchaeota* (Francis et al. 2007, Kowalchuk and Stephen 2001). Ammonia oxidizers have more restricted physiological characteristics and ecological niches, while denitrifiers have physiologies that can tolerate a range of environmental conditions and are likely to be broadly distributed across a variety of habitats (Francis et al. 2007, Goddard and Bradford 2003, Kowalchuk and Stephen 2001, Rich and Myrold 2004, Wallenstein et al. 2006). Physiological differences between microbial functional groups could contribute to the differential sensitivity of communities to oxygen/soil moisture levels in the environment

(Jayakumar et al. 2009). I hypothesized that ammonia oxidizers will be more sensitive to changes in soil moisture compared to denitrifiers because of their restricted physiological characteristics.

For denitrification to occur, anoxic conditions and nitrate are required. Nitrate serves as the terminal electron acceptor and an electron donor are necessary. Limited carbon availability, low temperatures, and low pH have been identified as factors that limit denitrification activity. Low temperatures and carbon availability specifically suppressed nitrous oxide reductase activity (Dendooven and Anderson 1995, Henderson et al. 2010, Holtan-Hartwig et al. 2002). In addition, denitrifier gene abundance (*nirS* and *nosZ*) has been shown to increase due to carbon amendments in an agricultural soil (Henderson et al. 2010). Denitrifying communities from successional fields have been shown to be more sensitive to changes in soil pH than communities in agricultural soils; oxygen inhibited denitrification enzyme activity more strongly in denitrifying communities in agricultural compared to successsional field soil (Cavigelli and Robertson 2000).

Nitrification activity is mainly influenced by pH and oxygen availability. Soil pH above 6.5 results in ammonia (NH_3) being dominant and is more easily taken up by microbial cells via diffusion. Below pH 6.5, the ionized NH_4^+ form requires energy-dependent transport to enter the cell, making it more energy-intensive to carry out nitrification (Prosser 2007). Nitrification has also been measured along a range of acidic to alkaline conditions. Different archaeal and bacterial ammonia oxidizer taxa were identified along a pH gradient from 4.9 to 7.5 (Nicol et al. 2008). In addition, higher nitrification rates were detected around plant root zones in anoxic sediments due to oxygen release by plants (Bodelier et al. 1996). Particular ammonia oxidizers are capable of tolerating anaerobic conditions, and nitrifying capacity can also be supported in fluctuating oxic and anoxic environments (Bodelier et al. 1996).

OVERVIEW OF STATISTICAL METHODS

Permutational (non-parametric) multivariate analysis of variance (PERMANOVA)

Permutational (non-parametric) multivariate analysis of variance (PERMANOVA) was used to assess the contribution of different environmental factors to community variation (McArdle and Anderson 2001). The adonis function in the R Statistics Package was used to carry out PERMANOVA (R Development Core Team 2011), and this method is analogous to redundancy analysis (Legendre and Anderson 1999). PERMANOVA, like nested analysis of molecular variance (AMOVA), is based on the sums of squared deviations whereby semimetric and metric distance matrices are partitioned among difference sources of variation (explanatory variables included in the model). PERMANOVA was used to partition sums of squares from a centroid based on a Bray-Curtis dissimilarity matrix, a semimetric distance matrix. This analysis is unaffected by assumptions associated with parametric MANOVA such as multivariate normality. Significance tests were based on 1000 permutations of the raw data and subsequent calculation of *F*-tests to create a pseudo *F*-distribution, analogous to using Fisher's *F*-ratio (McArdle and Anderson 2001). The *P*-value for each factor in the PERMANOVA model was calculated by comparing the observed *F*-value to the distribution of *F*-values obtained from 1000 permutations of the raw data. Factors in the PERMANOVA (effect) were considered significant if the observed *F*-value was greater than 95% of the *F*-values calculated from permutations (McArdle and Anderson 2001). The reported R^2 value is the treatment sums of squares/total sums of squares for each factor in the model. The adonis function carries out sequential tests (similar to Type I sums of squares) (Okansen et al. 2010). Only the effect of the last variable entered into the model, which is assessed after all other variables were accounted for, was included in the final PERMANOVA model summary to achieve Type III sums of squares to

obtain the variation due to an effect in the model after accounting for all other effects in the model.

Analysis of similarity (ANOSIM)

Analysis of similarity (ANOSIM) was used to test for significant differences in community composition among locations along the environmental gradient for microbial and plant communities. An ANOSIM R statistic was generated based on comparison of rank similarity within and among groups of samples, and significance of the group dissimilarity was based on permutation tests. An R-value of 1 indicates complete dissimilarity among groups and R = 0 indicates a high degree of community similarity among locations along the gradient.

Nonmetric multidimensional scaling (NMDS)

Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship among microbial communities along the moisture gradient. Nonmetric multidimensional scaling is based on the rank order relation of dissimilarities based on the Bray-Curtis similarity coefficient. The largest distance between points on the NMDS ordination represents the most dissimilar microbial communities.

OVERVIEW

To assess the relationships between environmental conditions and microbial community composition and activity in wetland ecosystems, I addressed the following questions in my dissertation: (i) what environmental filters are important to microbial communities at the local and landscape scales?; (ii) how do differences in soil factors affect microbial structure-function relationship in wetland ecosystems?; and (iii) does environmental history constrain microbial function under current drying/flooding disturbances? To address these questions, I used a

combination of observational and experimental approaches. I compared community composition across different restored wetlands and then focused on within-wetland differences in environmental factors and microbial community composition and activity. I also experimentally manipulated soil moisture conditions to compare the influence of prior hydrologic history on the response of microbially-mediated nitrogen cycling to short-term drying/flooding disturbance. By addressing these questions, I identified generalizable microbial responses and site-specific microbial responses to environmental filters.

The concept of environmental filtering is often used as a theoretical framework applied in restoration ecology, whereby environmental factors are identified to influence microbial distribution over space and time (Harper 1977, Holyoak et al. 2005, Zobel 1992). In Chapter 2 – *Environmental filters at dissimilar spatial scales influence plant and microbial communities in restored wetlands*, I investigated the relationship between landscape factors (climate, surrounding land use) and local soil factors (e.g., soil moisture, organic matter, pH) and plant and microbial communities in 27 restored wetlands. A range of plant and microbial community responses was expected to occur at the restored wetlands. Identification of scale and type of environmental factors acting on above and belowground communities in this study provides a way to monitor restoration of specific plant- and microbially-mediated wetland functions.

From the restored wetlands survey in Chaper 2, I identified that local soil factors such as soil moisture, redox conditions and nitrogen availability contributed to variation in microbial community composition. It is known that the influence of the local environment can mediate changes in community structure and function (Dumbrell et al. 2010, Gutknecht et al. 2006, Ikenaga et al. 2010, Pett-Ridge and Firestone 2005, Rousk et al. 2010, Swan et al. 2010). Variation in microbial functional response can be attributed to individual taxon-response ranging

in tolerance along a moisture gradient. In Chapter 3 – *Distribution of denitrifying and ammonia oxidizing microorganisms along a moisture gradient*, I aimed to assess the distribution of functional guilds capable of carrying out denitrification and nitrification. Microbial assemblages were studied with respect to a characterized environmental gradient in order to more specifically assess the relationship between the local environment and microbial communities. I compared soil denitrifier and ammonia oxidizer community composition along a moisture gradient in a restored floodplain wetland. Denitrifiers and ammonia oxidizers have contrasting responses to abiotic soil factors such as oxygen concentration and moisture. I also examined taxon-level patterns of denitrifiers and ammonia oxidizers to understand potential ecological mechanisms contributing to changes in community composition. Meeting my prediction, denitrifier taxa were observed to have a wider habitat distribution over the moisture gradient than ammonia oxidizers. Taxon-level differences contributed to observed changes in microbial community composition along the moisture gradient. Evaluation of denitrifier and ammonia oxidizer community composition along the same environmental gradient provides a direct comparison of microbial communities involved in nitrogen cycling in wetland ecosystems.

Environmental filters can also affect the relationship between microbial structure and function. In Chapter 4 – *Abiotic correlates of microbial community structure and function vary within wetlands*, I examined microbial community composition and activity associated with denitrification and nitrification within restored and natural wetlands. It is hypothesized that the relationship between microbial community composition and function are context dependent with respect to both function and the environment. Denitrifier and ammonia oxidizer community composition differed between upland and wetland plots at all sites, and were relatively constant through time, whereas microbial function decreased over time at most wetlands. Potential

denitrification and nitrification rates were mainly influenced by environmental conditions Neither community composition nor denitrifier/ammonia oxidizer gene abundance strongly influenced potential nitrification and denitrification rates. When focusing on the affect of community composition alone, nitrification was influenced by community composition more than denitrification. Microbial structure-function relationships varied in sensitivity to environmental change or disturbance.

In Chapter 5 – *Hydrologic history constrains wetland microbial structure and function under experimental drying/wetting regimes*, I experimentally manipulated soil moisture conditions to explore the influence of moisture on microorganisms involved in denitrification and nitrification. This experiment was designed to directly test for the influence of soil moisture on microbial community structure and function. I also examined the sensitivity of microbial nitrogen cycling to contemporary changes in drying/flooding events by comparing the response of microbes adapted to upland compared to wetland conditions. Experimental manipulation of soil moisture conditions allowed the comparison of microbial community composition and activity associated with denitrification and nitrification. Potential denitrification rate was more sensitive to drying/flooding treatments than was the potential nitrification rate. Denitrifier and ammonia oxidizer community assemblages significantly differed according to hydrologic history, but microbial communities did not significantly change in response to drying/flooding treatments. Based on this study, microbial functional response can vary in sensitivity to contemporary drying/flooding, influencing nitrogen cycling functions in restored wetlands.

SUMMARY

Changing land use from managed, arable systems to restored wetland ecosystems can improve ecosystem functions that combat nitrate pollution. These processes can only be carried out if microbial communities responsible for nutrient transformations are present and the environmental conditions are adequate for activity to proceed (Bedard-Haughn et al. 2006, Francis et al. 2005, Nogales et al. 2002, Norton et al. 2002, Wallenstein et al. 2006). Fluctuations in water levels (and the subsequent fluctuations in oxygen levels) will affect the balance between contrasting anaerobic and aerobic microbial processes such as denitrification and nitrification, respectively. The outcome of this balancing act will ultimately influence the nutrient content of the overlying waters. In this dissertation, I have contributed to the understanding of the dynamics between anaerobic and aerobic processes under changing environmental conditions (e.g., redox conditions, presence of plants, soil fertility), providing useful information to guide re-establishment of wetland water quality function.

REFERENCES

Bedard-Haughn, A., A. L. Matson, and D. J. Pennock. 2006. Land use effects on gross nitrogen mineralization, nitrification, and N2O emissions in ephemeral wetlands. Soil Biology & Biochemistry **38**:3398-3406.

Bodelier, P. L. E., J. A. Libochant, C. Blom, and H. J. Laanbroek. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Applied and Environmental Microbiology **62**:4100-4107.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology **81**:1402-1414.

David, M. B. and L. E. Gentry. 2000. Anthropogenic inputs of nitrogen and phosphorus and riverine export for Illinois, USA. Journal of Environmental Quality **29**:494-508.

Dendooven, L. and J. M. Anderson. 1995. Maintenance of denitrification potential in pasture soil following anaerobic events. Soil Biology & Biochemistry **27**:1251-1260.

Dumbrell, A. J., M. Nelson, T. Helgason, C. Dytham, and A. H. Fitter. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME Journal **4**:337-345.

Duncan, C. P. and P. M. Groffman. 1994. Comparing microbial parameters in natural and constructed wetlands. Journal of Environmental Quality **23**:298-305.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal **1**:19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America **102**:14683-14688.

Goddard, M. R. and M. A. Bradford. 2003. The adaptive response of a natural microbial population to carbon- and nitrogen-limitation. Ecology Letters **6**:594-598.

Gutknecht, J. L. M., R. M. Goodman, and T. C. Balser. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil **289**:17-34.

Harper, J. L. 1977. Population biology of plants. Academic Press, London.

Hartman, W. H., C. J. Richardson, R. Vilgalys, and G. L. Bruland. 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proceedings of the National Academy of Sciences of the United States of America **105**:17842-17847.

Henderson, S. L., C. E. Dandie, C. L. Patten, B. J. Zebarth, D. L. Burton, J. T. Trevors, and C. Goyer. 2010. Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions, and denitrification in anoxic soil microcosms amended with glucose and plant residues. Applied and Environmental Microbiology **76**:2155-2164.

Holtan-Hartwig, L., P. Dorsch, and L. R. Bakken. 2002. Low temperature control of soil denitrifying communities: Kinetics of N2O production and reduction. Soil Biology & Biochemistry **34**:1797-1806.

Holyoak, M., M. A. Leibold, and R. D. Holt. 2005. Metacommunities: Spatial dynamics and ecological communities. The University of Chicago Press, Chicago.

Hooper, D. U., D. E. Bignell, V. K. Brown, L. Brussaard, J. M. Dangerfield, D. H. Wall, D. A. Wardle, D. C. Coleman, K. E. Giller, P. Lavelle, W. H. Van der Putten, P. C. De Ruiter, J. Rusek, W. L. Silver, J. M. Tiedje, and V. Wolters. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. Bioscience **50**:1049-1061.

Hunter, R. G. and S. P. Faulkner. 2001. Denitrification potentials in restored and natural bottomland hardwood wetlands. Soil Science Society of America Journal **65**:1865-1872.

Ikenaga, M., R. Guevara, A. L. Dean, C. Pisani, and J. N. Boyer. 2010. Changes in community structure of sediment bacteria along the Florida Coastal Everglades marsh-mangrove-seagrass salinity gradient. Microbial Ecology **59**:284-295.

Jayakumar, A., G. D. O'Mullan, S. W. A. Naqvi, and B. B. Ward. 2009. Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. Microbial Ecology **58**:350-362.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55**:485-529.

Legendre, P. and M. J. Anderson. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs **69**:1-24.

Luton, P. E., J. M. Wayne, R. J. Sharp, and P. W. Riley. 2002. The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. Microbiology-Sgm **148**:3521-3530.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology **82**:290-297.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology **10**:2966-2978.

Nogales, B., K. N. Timmis, D. B. Nedwell, and A. M. Osborn. 2002. Detection and diversity of expressed denitrification genes in estuarine sediments after reverse transcription-PCR amplification from mRNA. Applied and Environmental Microbiology **68**:5017-5025.

Norton, J. M., J. J. Alzerreca, Y. Suwa, and M. G. Klotz. 2002. Diversity of ammonia monooxygenase operon in autotrophic ammonia-oxidizing bacteria. Archives of Microbiology **177**:139-149.

Panno, S. V., W. R. Kelly, K. C. Hackley, H. H. Hwang, and A. T. Martinsek. 2008. Sources and fate of nitrate in the Illinois River Basin, Illinois. Journal of Hydrology **359**:174-188.

Peralta, A. L., J. W. Matthews, and A. D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology **76**:4207-4215.

Pett-Ridge, J. and M. K. Firestone. 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Applied and Environmental Microbiology **71**:6998-7007.

Prosser, J. I. 2007. The Ecology of Nitrifying Bacteria.*in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle. Elsevier, Oxford.

Rabalais, N. N., R. E. Turner, and D. Scavia. 2002. Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. Bioscience **52**:129-142.

Reed, H. E. and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology **62**:161-170.

Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology **69**:5974-5982.

Rich, J. J. and D. D. Myrold. 2004. Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. Soil Biology & Biochemistry **36**:1431-1441.

Rousk, J., E. Baath, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal **4**:1340-1351.

Schimel, J. P. and J. Gulledge. 1998. Microbial community structure and global trace gases. Global Change Biology **4**:745-758.

Schneider, D. W. 1996. Enclosing the floodplain - Resource conflict on the Illinois River, 1880-1920. Environmental History **1**:70-96.

Stokstad, E. 2008. Environmental regulation: New rules on saving wetlands push the limits of the science. Science **320**:162-163.

Swan, B. K., C. J. Ehrhardt, K. M. Reifel, L. I. Moreno, and D. L. Valentine. 2010. Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. Applied and Environmental Microbiology **76**:757-768.

Wallenstein, M. D., D. D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications **16**:2143-2152.

Wardle, D. A., H. A. Verhoef, and M. Clarholm. 1998. Trophic relationships in the soil microfood-web: predicting the responses to a changing global environment. Global Change Biology **4**:713-727.

Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster, G. M. Wimp, D. G. Fischer, J. K. Bailey, R. L. Lindroth, S. Woolbright, and C. R. Kuske. 2003. Community and ecosystem genetics: A consequence of the extended phenotype. Ecology **84**:559-573.

Zak, D. R., C. B. Blackwood, and M. P. Waldrop. 2006. A molecular dawn for biogeochemistry. Trends In Ecology & Evolution **21**:288-295.

Zedler, J. B. 2003. Wetlands at your service: reducing impacts of agriculture at the watershed scale. Frontiers In Ecology And The Environment **1**:65-72.

Zobel, M. 1992. Plant species coexistence—the role of historical, evolutionary and ecological filter. Oikos **65**:314-320.

FIGURES

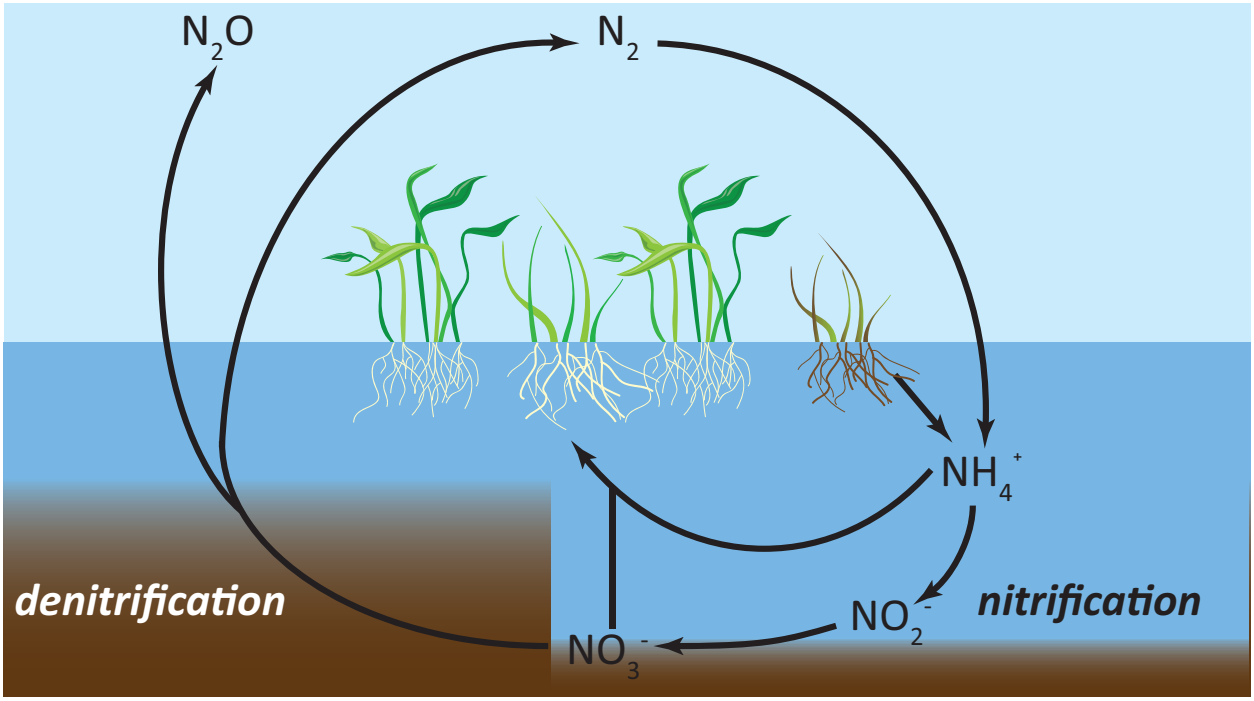


Figure 1.1. Simplified schematic of nitrogen cycling transformations in wetlands. Denitrification and nitrification are key transformations that contribute to wetland nitrogen cycling. Different microbial populations are capable of carrying out various nitrogen cycling transformations.

CHAPTER 2

ENVIRONMENTAL FILTERS AT DISSIMILAR SPATIAL SCALES INFLUENCE PLANT AND MICROBIAL COMMUNITIES IN RESTORED WETLANDS

ABSTRACT

A community assembly framework has been used in the context of ecological filters to inform wetland restoration. Ecological filters include abiotic and biotic factors that can potentially influence community composition. Identifying environmental factors that contribute to plant and microbial community variation at different spatial scales can provide critical information to aid in monitoring plant and microbial communities and ultimately, the ecosystem services they provide, in restored wetlands. The objectives of this study were to (i) quantify the influence of landscape (e.g., surrounding land use, precipitation) and local soil factors (e.g., soil organic matter, pH, moisture) on plant, bacterial and denitrifier communities and (ii) assess the spatial relationships between environmental and biological community matrices in 27 restored Illinois wetlands. We hypothesized that plant and microbial community composition are sensitive to different environmental filters operating at dissimilar spatial scales. To examine the relationship between landscape and local factors on plant and microbial community composition, we surveyed plant and total bacterial and denitrifier community composition along with landscape (surrounding land use, regional climate variables) and local soil factors (soil organic matter, moisture, inorganic nitrogen, pH, redox status) at 27 restored wetlands in Illinois. The contribution of environmental factors to plant and microbial community variability was determined using permutational multivariate analysis of variance (PERMANOVA). Plant communities were influenced by the proportion of developed land surrounding the wetland,

temperature (growing degree-days) and local soil fertility, specifically nitrogen availability. In contrast, microbial communities were influenced by local soil factors, especially soil pH. Bacterial and denitrifier communities differed along a range of redox conditions and inorganic N availability within restored wetlands. In addition, the correlation between total bacterial communities and the matrix of environmental variables ($r_M = 0.06461$, $P = 0.001$) was relatively strong compared to environment-denitrifier ($r_M = 0.2467$, $P = 0.036$) or environment-plant ($r_M = 0.2384$, $P = 0.003$) relationships. Our results demonstrate that different landscape and local soil factors influence plants and microorganisms. These findings provide a means to assess the potential for restoring plant and microbially mediated wetland functions (i.e., habitat provision, nutrient cycling).

INTRODUCTION

Ecological filtering, a process by which environmental factors structure communities, is a common theoretical framework applied in restoration ecology (Harper 1977, Holyoak et al. 2005, Zobel 1992). Initially, species must pass through the abiotic filter and tolerate chemical and physical conditions for successful establishment. Given maintenance of life support processes, species must successfully interact with the established species pool in order to persist (Fattorini and Halle 2004, Naeem et al. 1999). Ecological filtering occurs at multiple spatial scales and can differentially structure plant and soil microbial communities (Burton et al. 2011, Getzin et al. 2008, Horner-Devine et al. 2007, Keddy 1992).

Plant community composition within a given restoration site can be affected by abiotic and biotic filters at landscape and local spatial scales. Climatic factors such as rainfall and temperature are considered abiotic filters that influence plants on a regional scale (Zobel 1992).

Landscape-level filters, such as surrounding land use and hydrology, impact plant distribution indirectly by affecting local inputs of nutrients, soil moisture and water availability (Gemborys and Hodgkins 1970, Keddy 2000, Mitsch and Gosselink 2007, Wright et al. 2003). Substrate availability, encompassing soil fertility, water availability, and toxicity levels at the local scale, has also been considered a set of abiotic filters that can directly affect plants (Hobbs and Norton 2004). Specifically, soil fertility can influence plant community structure by way of resource partitioning among plants (Bontti et al. 2011, Harpole and Suding 2011, Hobbs and Norton 2004, Weiher and Keddy 1995). Besides plant-plant interactions, positive and negative plant-microbe interactions have also been identified as important in shaping plant communities (Bever et al. 1997, Reynolds et al. 2003). On a local scale, microbes influence plant growth and reproduction through solubilization of mineral nutrients in soil (Barness et al. 1991), N_2 fixation (Reed et al. 2010), plant growth promotion (Lugtenberg and Kamilova 2009), and plant pathogen suppression (Bever et al. 1997, Thomashow and Weller 1990). Soil microorganisms suppressing plant growth through competition for nutrients (Schimel and Bennett 2004) and inhibiting plant growth by soil-borne pathogens are only a few examples of negative plant-microbe interactions. Together, a combination of landscape- and local-scale environmental filters can contribute to plant community composition.

Like plants, landscape- and local-scale environmental filters also shape soil microbial communities. At the landscape scale, microbes are influenced by climate, surrounding land use and watershed-scale hydrology, mediated through local scale processes. For example, physical and chemical conditions in the soil can alter transport of solutes resulting in changes in substrate availability for soil microbes (Poll et al. 2006). Poll et al. (2006) demonstrated that bacteria and fungi used different substrate utilization strategies in the interface between the soil surface and

plant litter. Bacteria took up more substrates from the soil associated with transport processes, and fungi utilized substrates and incorporated carbon directly from plant litter (Poll et al. 2006). In addition, local-scale abiotic and biotic filters have been correlated with microbial community composition. Previous studies identified soil factors such as pH, moisture, and nutrient availability important for contributing to variation in microbial communities. Specifically, soil pH has been identified in many studies as a significant factor influencing microbial communities at multiple spatial scales (Bru et al. 2011, Fierer and Jackson 2006, Hartman et al. 2008). Soil pH is a factor that affects substrate availability, which might be a possible mechanism contributing to strong microbial responses to pH gradients. In addition, fluctuations in soil moisture can result in shifts in microbial community composition as a response of varied microbial tolerance (Truu et al. 2009). Hydrologic changes in the soil environment can affect oxygen and nutrient availability, influencing soil redox status and contributing to habitat variability for soil microorganisms (Pett-Ridge and Firestone 2005, Picek et al. 2000). Changes in soil redox status influence the pool of electron acceptors available to microorganisms, resulting in different microorganisms being adapted to static or dynamic redox conditions (DeAngelis et al. 2010). Our ability to prediction restoration of microbial functions such as water quality improvement via denitrification can be enhanced through understanding the relative influence of landscape compared to local factors on soil microbial communities.

In the present study, we aimed to identify potential environmental factors that contribute to variation in plant and microbial communities for monitoring restored wetlands. Using a survey approach, we assessed the relationships between landscape and local environmental factors and plant and microbial communities at 27 restored wetlands in Illinois, USA. We were specifically interested in (i) quantifying the influence of specific landscape and local soil factors on plant,

total bacterial and denitrifier community composition and (ii) assessing spatial relationships between environmental and biological communities by correlating environmental and biological community matrices. We hypothesized that plant and microbial communities are affected by environmental factors that vary at different spatial scales. We predicted that local factors would more strongly structure microbial communities, whereas both local and landscape factors would influence plants communities. Tracking community composition related to plant and microbial-based wetland functions (i.e., habitat provisioning, biomass production, nutrient cycling) can further our understanding of how environmental drivers induce changes in plant and microbial wetland communities.

MATERIALS AND METHODS

Overview of approach

We described three different assemblages in restored wetlands (plants, total bacterial, denitrifiers). Environmental variables were measured at three scales (landscape, local among wetlands, local within wetlands) (Table 2.1). Composition of each assemblage was then related to (A) environmental predictors at the different spatial scales, and (B) the other assemblages (e.g., plant-bacteria, plant-denitrifiers) and environmental conditions (e.g., bacteria-environment).

To address the objectives, (i) landscape and local soil factors were predictors for plant, bacterial and denitrifier communities in permutational MANOVA (PERMANOVA) to test for the effect of environmental factors on communities; and (ii) the relationship between plant and microbial communities to each other and to the matrix of environmental variables was carried

out using Mantel tests to relate biological communities and environmental conditions based on dissimilarity and distance matrices, respectively.

Study Sites

A total of 27 restored wetlands located from 37°17' to 42°27' latitude and 87°53' to 91°20' longitude were included in this study (Fig. 2.1). The sites were established between 1992 and 2002 by the Illinois Department of Transportation in exchange for wetlands impacted during road construction (Matthews 2008, Matthews et al. 2009). Wetlands were constructed by changing hydrologic conditions through removal of tile drains, excavation and were refilled with topsoil and/or construction of berms or weirs. The size of each site ranged from 0.11 ha to 7.07 ha. Wetland sites were previously described in full detail (Matthews 2008, Matthews et al. 2009) (Table A.1).

Landscape factors (among wetlands)

At the landscape-scale, wetland context was described by land cover within 1000 m and regional climate descriptors. We quantified percent open water, wetland (including forested wetlands), developed land, upland forest, and cropland (agricultural land) within a 1000-m radius around each site using ArcGIS 9.0 and data from the Illinois Gap Analysis Land Cover database (Luman et al. 2004). Based on major land cover gradients in Illinois, wetland (combined open water and wetland), developed land, and cropland were included in our analyses. Climate data (precipitation and temperature) were summarized from the nearest climate monitoring station provided by the Midwestern Regional Climate Center (http://mrcc-isws-illinois-edu) (Matthews et al. 2009). For each wetland, average growing degree-days (GDD; base 10°C) was compiled from 1990-2000 data and annual precipitation totals were compiled from 1971-2000 data provided by the National Climate Data Center (Matthews et al. 2009).

Local soil factors (among and within wetland)

At each site, a total of eight 0.25-m^2 quadrats were placed randomly along four transects spanning the entire wetland (n = 8). Soil samples representing a composite of eight soil cores, 12-cm deep and 1.9-cm diameter, were collected from each quadrat. The pH of the soil solution (1:1 soil:water) was determined for each composite sample, and available ammonium (NH_4^+) and nitrate (NO_3^-) were analyzed using colorimetric analyses based on the Berthelot method (Rhine et al. 1998, Sims et al. 1995). We analyzed soil organic matter content (total organic C and total N) using combustion methods (ECS 4010, COSTECH Analytical Instruments, Valencia, CA, USA).

Indicator of Reduction in Soils (IRIS) tubes (InMass Technologies, West Lafayette, IN, USA) were installed in four quadrats at each wetland for approximately two weeks. Locations of IRIS tubes were chosen to represent the range of site hydrologic conditions. Under saturated, reducing conditions, the Fe^{3+} in iron oxide-based (ferrihydrite) paint on the tubes is reduced to Fe^{2+} via microbial oxidation of organic matter, the iron and enters solution, and thus, the IRIS tubes are indicative of site hydrology (Castenson and Rabenhorst 2006, Jenkinson and Franzmeier 2006). Digital images of the IRIS tubes were taken and transformed to black/white pixels to quantify loss of ferrihydrite paint. The amount reduced (white area) per unit time of field incubation was used as a proxy for soil redox status. The amount of paint reduced was quantified as the percent of white to total pixels on the IRIS tube. Image analysis to obtain IRIS tube reduction (percent white pixels) was accomplished by using Adobe Photoshop CS4.

The minimum, maximum and range of landscape- and local-scale factors are summarized in Table A.2.

Plant community data

Vegetation was surveyed in the established 8 quadrats at 27 restored wetlands in 2006. All vascular plant species observed in each quadrat were assigned a cover class (<1%, 1–5%, 6–25%, 26–50%, 51–75%, 76– 95%, or 96–100%) to assess plant community composition at the plot-level. Further details regarding vegetation sampling are found in Matthews et al (2009). Previous analysis of local- and landscape- factors on plant assemblages has been published (Matthews et al. 2009).

In the present study, analysis of plant and microbial communities in parallel adds significant additional insight into how plants and microorganisms respond to the same environmental conditions, allowing for comparison among multiple taxon assemblages. At the wetland-level, plant and microbial community composition were represented by the average relative abundance of each plant species across the eight quadrats. The full plant and microbial community matrix (8 quadrats per wetland) were used as a response matrix to assess the influence of landscape and local wetland factors on plant communities (using PERMANOVA).

M**icrobial community data**

Total genomic DNA was extracted from freeze-dried soil samples using the FastDNA SPIN Kit for Soil (MP Biomedicals). Genomic DNA was purified using a cetyl trimethyl ammonium bromide (CTAB) extraction to remove contaminating humic acids (Sambrook and Russell 2001). Bacterial community composition in wetland soil was assessed using terminal restriction fragment length polymorphism (T-RFLP) (Liu et al. 1997). PCR reactions to amplify 16S rRNA genes for T-RFLP analysis contained PCR buffer consisting of 50 mM Tris (pH 8.0), 250 μg of bovine serum albumin per ml and 3.0 mM $MgCl_2$ (Idaho Technology Inc., Salt Lake City, UT), 250 μM of each dNTP, 10 pmol of each primer, 1.25 U of Taq polymerase (Promega,

Madison, WI), and 20 ng of extracted DNA in a final volume of 25 µl. The 16S rRNA genes were amplified using primers 8F, 5'-AGAGTTTGATCMTGGCTCAG-3' (bacteria-specific, 16S rRNA gene) and 1492R, 5'-GGYTACCTTGTTACGACTT-3' (universal, 16S rRNA gene) (Lane 1991). The 8F primer was labeled with the phosphoramidite dye 6-FAM. Reactions were cycled with an initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 35 sec, 55°C for 45 sec, and 72°C for 2 min, with a final extension carried out at 72°C for 2 min. PCR products from each sample were digested in single-digests containing *Hha*I, *Msp*I or *Rsa*I.

T-RFLP analysis was also used to target the functional gene *nosZ*, which encodes the catalytic subunit of nitrous oxide reductase, the enzyme that catalyzes the final step in denitrification (Rich et al. 2003, Zumft 1997). PCR reactions contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of Taq polymerase (Promega), and 100 ng of extracted DNA in a final volume of 50 µl. The 700 bp *nosZ* gene was amplified using forward primer (*nosZ*-F-1181), 5'-CGCTGTTCITCGACAGYCAG-3' for the reverse primer (*nosZ*-R-1880) 5'-ATGTGCAKIGCRTGGCAGAA-3' (Rich et al. 2003). The *nosZ* reverse primer was labeled with the phosphoramidite dye 6-FAM. Reactions were cycled with initial denaturation at 94°C for 3 min, followed by 25 cycles at 94°C for 45 s, 56°C for 1 min, and 72°C for 2 min, with a final extension carried out at 72°C for 7 min. The Qiagen MinElute PCR purification kit was used to combine 3 PCR reactions and concentrate *nosZ* PCR product, and each sample was digested in a single-digest containing *Alu*I, *Hha*I or *Mbo*I.

The length and abundance of terminal restriction fragments (T-RF) were assessed by denaturing capillary electrophoresis using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 63°C and 15 kV with a run time

of 120 min using POP-7 polymer. A custom 100- to 2000-bp Rhodamine X-labeled size standard (Bioventures, Murfreesboro, TN) was used as the internal size standard for each sample for 16S T-RFLP analysis, and the ABI GeneScan ROX 1000 size standard (Applied Biosystems, Foster City, CA) was used as the internal size standard for the *nosZ* T-RFLP analysis.

T-RFLP data were analyzed using GeneMarker version 1.6 (SoftGenetics, State College, PA). To improve the signal to noise ratio, a second-derivative transformation was applied to the raw data prior to size-calling and alignment. To include the maximum number of peaks while excluding background fluorescence, a fluorescence cutoff of 100 fluorescence units was used. The signal strength (peak area) of each T-RF peak was normalized to account for run-to-run variations in signal detection by dividing the area of individual peaks by the total fluorescence (area) detected in each profile, expressing each peak as a proportion of the total fluorescence (relative abundance) for each digest (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Relative fluorescence data derived from each digest were concatenated for each sample prior to statistical analyses. At the wetland-level, microbial community composition was represented by averaging the relative abundance of each T-RF across the eight quadrats.

Statistical analyses

Permutational (non-parametric) multivariate analysis of variance (PERMANOVA) was used to assess the contribution of different environmental factors to community variation (McArdle and Anderson 2001). Pearson's correlation was computed between all landscape variables, and average growing degree-days and temperature were highly correlated ($r = 0.9820$) so temperature was removed prior to PERMANOVA. Calculation of Pearson's correlation between all soil factors revealed that TOC and TN were highly correlated ($r = 0.8433$), so TN was removed from the PERMANOVA model. A list of predictor variables used according to

spatial scale is summarized in Table 2.1. The adonis function in the R Statistics Package was used to carry out PERMANOVA (R Development Core Team 2011). PERMANOVA was used to partition sums of squares from a centroid based on a Bray-Curtis dissimilarity matrix, a semimetric distance matrix. This analysis is unaffected by assumptions associated with parametric MANOVA such multivariate normality, homogeneity of covariance matrices, and independence of observations. Because of the experimental design of this study, the adonis function allowed us to test for the plot-level contribution of the local soil factors on plant and microbial communities and the contribution of plant types on soil microbial communities by restricting the permutation of plot-level data to plots within a wetland using the strata argument. Significance tests were based on 1000 permutations of the raw data and subsequent calculation of *F*-tests to create a pseudo *F*-distribution, analogous to using Fisher's *F*-ratio (McArdle and Anderson 2001). The *P*-value for each factor in the PERMANOVA model was calculated by comparing the observed *F*-value to the distribution of *F*-values obtained from 1000 permutations of the raw data. Factors in the PERMANOVA (effect) were considered significant if the observed *F*-value was greater than than 95% of the *F*-values calculated from permutations (McArdle and Anderson 2001). The reported R^2 value is the treatment sums of squares/total sums of squares for each factor in the model. The adonis function carries out sequential tests (similar to Type I sums of squares) (Okansen et al. 2010). Only the effect of the last variable entered into the model, which is assessed after all other variables were accounted for, was included in the final PERMANOVA model summary.

To analyze potential interactions between regional climate variables known to influence local scale factors, each wetland was categorized into North, Central, or South region to reflect regional differences throughout the state. Permutation in the adonis function was restricted to

within region (strata = region). This permutation strategy was used to test for regional climate effects potentially interacting with local soil effects. Local soil factors found to significantly contribute to community variation were the same when regional climate variables were incorporated in or removed from the PERMANOVA model. The final PERMANOVA models included soil factors considered at the wetland- and plot-levels did not incorporate climate variables.

To relate plant and microbial communities to each other and to the matrix of environmental variables, two dissimilarity or dissimilarity and distance matrices were compared using a series of Mantel tests (Legendre and Legendre 1998). Similarity matrices based on Bray-Curtis were separately computed for the plant, bacterial and denitrifier communities. Euclidean distance matrices were computed separately for the set of soil variables and landscape-level factors at each site. Environmental variables were standardized prior to creation of distance matrices. Similarity and distance matrices were computed at the wetland level and at the plot level for comparison. The Mantel test statistic (r_M) based on Pearson's product-moment correlation was generated between similarities or distances between each wetland or plot. Separate Mantel tests were run for all combinations of dissimilarity matrices and dissimilarity-distance matrices using the mantel function in the R Statistics Package (R Development Core Team 2011).

RESULTS

Relationship between environmental factors and plant communities at different spatial scales

Wetland-level (among wetlands) variables explained more variation in both plant and microbial assemblages than the plot-level (within wetlands) variables (Tables 2.2-2.6). Based on the PERMANOVA results, the landscape level variables, the proportion of developed land surrounding a wetland and growing degree-days (GDD) significantly explained plant community variation (developed: $R^2 = 0.0624$, $P = 0.0030$; GDD: $R^2 = 0.0575$, $P = 0.0120$) (Table 2.2a). Inorganic N (nitrate and ammonium) significantly contributed to plant community composition at the wetland-level (nitrate: $R^2 = 0.0563$, $P = 0.0180$; ammonium: $R^2 = 0.0557$, $P = 0.0180$) (Table 2.2b). At the plot-level, inorganic N (nitrate and ammonium) significantly contributed to plant community variation (nitrate: $R^2 = 0.0251$, $P = 0.0040$; ammonium: $R^2 = 0.0187$, $P = 0.0010$) to a relatively small degree (Table 2.2c). In addition, redox status also significantly affected plant communities to a relatively weaker degree within wetlands (soil redox: $R^2 = 0.0126$, $P = 0.0140$) (Table 2.2c).

Relationship between environmental factors and bacterial communities at different spatial scales

A total of 698 terminal restriction fragments (T-RF's) represented the total bacterial communities from soils collected at 27 restored wetlands. Landscape factors related to water availability and temperature were important to bacterial communities. Both precipitation and growing degree-days significantly explained bacterial community variation (precipitation/precip: $R^2 = 0.0542$, $P = 0.0430$; GDD: $R^2 = 0.0694$, $P = 0.0100$); however, land use surrounding the wetlands was not as predictive for bacterial community composition (Table 2.3a). Soil pH and

redox status accounted for variation in bacterial communities at the wetland-level (pH: R^2 = 0.1083, P = 0.0010; redox status: R^2 = 0.0471, P = 0.0280) (Table 2.2b). In addition, soil C:N ratio also contributed to variation in bacterial community composition (R^2 = 0.0463, P = 0.0350) (Table 2.3b) at the wetland-level. At the plot-level, soil pH and redox status accounted for variation in bacterial communities (pH: R^2 = 0.0732, P = 0.0010; redox status: R^2 = 0.0201, P = 0.0350) (Table 2.3c). Ammonium, total organic carbon (TOC) and moisture were also important in potentially shaping bacterial communities to a small but significant degree (ammonium: R^2 = 0.0126, P = 0.0020; TOC: R^2 = 0.0171, P = 0.0010; moisture: R^2 = 0.0201, P = 0.0010) (Table 2.3c).

Relationship between environmental factors and denitrifier communities at different spatial scales

A total of 379 terminal restriction fragments (T-RF's) represented the denitrifier communities in soils collected from the 27 restored wetlands. Landscape factors did not significantly explain patterns in denitrifier community composition among the wetlands (Table 2.4a). Soil pH and total organic carbon accounted for variation in denitrifier communities at the wetland-level (pH: R^2 = 0.0705, P = 0.0030; TOC: R^2 = 0.0541, P = 0.0390) (Table 2.4b). At the plot-level, soil pH and total organic carbon accounted for variation in denitrifier communities (pH: R^2 = 0.0465, P = 0.0010; TOC: R^2 = 0.0260, P = 0.0010) (Table 2.4c). Soil moisture and C:N ratio also contributed to a small but significant amount of denitrifier variation at the plot-level (C:N ratio: R^2 = 0.0185, P = 0.0170; moisture: R^2 = 0.0278, P = 0.0010; ammonium: R^2 = 0.0088, P = 0.0320) (Table 2.4c).

Relationship between community and environment matrices

Weak correlations between biological communities and wetland-level landscape factors were observed (bacteria: $r_M = 0.2860$, denitrifier: $r_M = 0.1932$, plant: $r_M = 0.2887$) (Table 2.5a). Bacterial communities among and within wetlands were more strongly correlated with soil factors (wetland-level $r_M = 0.6461$, plot-level $r_M = 0.4706$) compared to the relationship between denitrifier communities and soil factors (wetland-level $r_M = 0.2467$, plot-level $r_M = 0.2513$) or plant communities and soil factors (wetland-level $r_M = 0.2384$, plot-level $r_M = 0.1312$) (Table 2.5b). Bacterial and plant communities (wetland-level $r_M = 0.3258$, plot-level $r_M = 0.2162$) or denitrifier and plant communities (wetland-level $r_M = 0.3048$, plot-level $r_M = 0.2158$) showed similar correlations (Table 2.5c). Overall, the relationship between bacterial communities and the described soil factors (within and among wetlands) was stronger than the relationship between plant communities and soil factors or the plants and bacterial communities.

DISCUSSION

Environmental filtering across space: landscape and local-scale abiotic factors influence community composition

Species from a regional pool can be locally selected by a set of environmental factors, resulting in a subset of the regional species pool capable of persisting within a given local environment. Previous studies have not directly compared the effect of local level factors on plant and microbial community composition at different spatial scales in restored wetlands (Drenovsky et al. 2010, Hartman et al. 2008, LeCoeur et al. 1997, Wright et al. 2003). In the present study, we examined the role of potential environmental filters on plant and microbial

communities by evaluating the contribution of landscape and local-level environmental factors on plant and microbial communities.

Plant communities. At the wetland-level, the present study showed that plant communities were structured by the proportion of developed land surrounding the wetland, a specific landscape factor that has been identified to influence available species pools and plant dispersal into wetlands (Zobel 1992). In addition, growing degree-days have been shown to affect plant community composition, which has been demonstrated to influence regional plant diversity in Canadian wetlands (Locky and Bayley 2010). In the present study, soil fertility characterized by inorganic N pools, significantly contributed to plant community variation at the wetland-level. Soil fertility has been previously shown to be an important factor shaping wetland plant communities, where wetland plant species were identified to have different nutrient limitations compared to the whole plant community (Bedford et al. 1999). Local hydrology, represented by plot-level redox status and soil moisture, was also correlated to plant communities in restored wetlands to a small but significant degree. Previous studies have also observed that hydrology is a determining factor for plant community composition and strongly influences their distribution in wetland ecosystems (Keddy 2000, Mitsch and Gosselink 2007).

The relative influence of local and landscape level variables on plant species composition in these restored wetlands has been described using constrained ordination and variance partitioning (Matthews et al. 2009). Both local and regional-scale factors were identified as important for shaping plant species composition in restored wetlands. To build on the Matthews et al. (2009) study, we took a multi-community approach to identify and compare potential abiotic filters on plant, bacterial, and denitrifier communities in restored wetlands and to compare the relative effect of environmental variables on different taxon assemblages. Our

results are broadly consistent with Matthews et al. (2009); plant communities may be shaped by landscape factors influencing dispersal, soil fertility (represented by inorganic N levels), and local hydrology (represented by redox status).

Bacterial communities. We did not find a significant relationship between land use surrounding wetlands and total bacterial community. However, the influence of previous land use has previously been demonstrated to significantly affect microbial community composition (Bru et al. 2011, Drenovsky et al. 2010, Enwall et al. 2010, Fraterrigo and Rusak 2008, Hartman et al. 2008). For example, previous studies have identified on-site land use as an important influence on bacterial communities, where different land use histories resulted in different bacterial communities (Bru et al. 2011, Drenovsky et al. 2010, Enwall et al. 2010, Fraterrigo and Rusak 2008, Hartman et al. 2008). In addition, growing degree-day was also accounted for variation in bacterial communities, and this may be related to changes in air temperature that could influence soil temperature. Changes in soil temperature have been identified as significant determinants of microbial community composition and activity compared among tropical, temperature, and taiga soils (Balser and Wixon 2009). In addition, we found that precipitation and moisture significantly explained bacterial variation within and among wetlands, respectively (Table 2.2a, 2.2b); whereas, a previous study demonstrated that soil moisture at the local-level was more important to shaping bacterial community composition than precipitation measured at the landscape-level (Drenovsky et al. 2010). For bacterial communities, temperature is a potentially important landscape-level filter, while soil moisture is an important filter at the local-level.

Changes in wetland hydrology occurs naturally or anthropogenically via management, where hydrology can influence local soil redox conditions resulting in recharge of oxygen and

nutrient pools. Variability in environmental conditions can lead to increased number of microhabitats in the soil which can support a higher bacterial diversity due to variations in bacterial tolerance to fluctuating redox conditions. For example, unique bacterial communities are able to adapt to fluctuating and stable redox conditions (DeAngelis et al. 2010, Truu et al. 2009). Bacterial communities may be influenced by redox conditions because some bacteria are able to persist only in particularly stable or dynamic hydrologic conditions; therefore, both dynamics and absolute redox conditions matter (DeAngelis et al. 2010, Mentzer et al. 2006, Schimel et al. 2007).

At the wetland-level, soil pH was significant in explaining patterns in microbial community composition in the present study and in several previous studies (Bru et al. 2011, Enwall et al. 2010, Fierer and Jackson 2006). Soil pH can indirectly affect microorganisms by altering nutrient availability and directly influence microorganisms that need specialized mechanisms to maintain near-neutral conditions when exposed to acidic or alkaline environmental conditions (Madigan and Martinko 2006). Soil pH is related to several soil properties and influences other properties such as nutrient availability. To a small but significant degree, soil organic matter (SOM) quality at the wetland-level (C:N ratio) shaped bacterial communities. Soil C:N ratio is influenced by plant inputs, and soil nutrient availability has been previously demonstrated to alter bacterial community composition (Bradford et al. 2008). Hydrology appears to be an important determinant of bacterial community structure at the wetland level, as indicated by the significant relationship between soil redox status and the bacterial community. Soil organic matter quantity at the plot-level was also important factor in structuring microbial communities. Previous studies identified SOM inputs as directly contributing to microbial metabolism and influencing carbon and nutrient cycling rates in soils

(Dijkstra et al. 2010, Hooper et al. 2000, Paterson et al. 2009, Stephan et al. 2000, Strickland et al. 2009). Landscape and local abiotic factors were both involved in shaping bacterial community composition.

Denitrifier communities. Similar to the total bacterial community, soil pH and soil organic matter both contributed to variability in denitrifier community composition. Soil pH has been shown to be a strong driver of microbial communities across many spatial scales (Bru et al. 2011, Fierer and Jackson 2006, Jones and Hallin 2010). However, contrary to effects on total bacteria, none of the landscape factors contributed to denitrifier community variation in the present study.

At the plot-level, soil pH, ammonium and redox status were important but had relatively weak effects on denitrifier community variation. In addition, denitrifier community composition was weakly correlated at the plot-level with redox status and moisture, factors that are influenced by the hydrology of the site.

Correlated patterns weakly detected between plants, microbes, and the environment

There was no strong evidence to suggest that community composition of plants and microbes were correlated (Table 2.7). The present results corroborate previous studies that identified individual plant species having stronger effects on microbial community composition compared to plant community effects as a whole (Kardol et al. 2010, Mitchell et al. 2010). In addition, weak correlations between communities and the environment may suggest that specific environmental variables, as opposed to the multivariable matrix of environment variables, have more power to explain community composition.

Understanding the influence of environmental factors on communities becomes more complicated as number of taxa increases. Microbial communities, in particular, are composed of

a subset of taxa that respond to measured environmental parameters, while other taxa do not respond (data not shown), resulting in low variance explained by some statistical models. Identifying environmental factors that significantly contribute to community composition is still biologically relevant and statistically significant. The statistical analyses used in this study allow identification of potential environmental factors that may be environmental filters for plant and microbial communities. Ultimately, experimental manipulation of environmental factors will allow for directly assessment of the strength of environmental filters on community composition.

Environmental filtering vs. community assembly history

Under the single stable equilibrium scenario, different communities develop from the regional species pool in response to different sets of environmental filters, and similar communities assemble in response to the same environment filters (Sommer 1991, Tilman et al. 1986). Environmental filtering is a potentially important process structuring plant and microbial communities in this study. Specifically, inorganic N levels influence plant community composition, and pH consistently affected microbial variation. Despite differences in surrounding land use that could influence invasion sequence of individuals to the local species pool, we identified local soil parameters as significantly contributing to plant and microbial community variation. Considering the strength that local environment has on supporting plant and microbial communities, choosing restoration sites based on soil conditions known to support particular plant or microbial communities could enhance restoration outcomes related to plant and microbially-mediated functions in restored wetlands.

ACKNOWLEDGEMENTS

This chapter was completed in collaboration with Dr. Jeffrey Matthews, Diana Flanagan, and Dr. Angela Kent. This material was supported by a National Great Rivers Research and Education Center grant to Anton Endress and J. W. Matthews and the University of Illinois Campus Research Board under Award Number 06351, and the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under project number ILLU 875-374 to A. D. Kent. This research was also supported by the Illinois Water Resources Center Research Assistantship to A. L. Peralta and A. D. Kent. Wetland sites were constructed by the Illinois Department of Transportation. Anton Endress, Patrick Baldwin and Arun Soni assisted with field work. Diane Szafoni and Ryan Lash provided GIS assistance. We thank Richard Lankau and Anthony Yannarell for statistical assistance. James Dalling, Yong Cao, Michelle Wander, Emily Wheeler, Richard Lankau, Chris Balakrishnan, and Sara Paver contributed helpful comments to earlier versions of this manuscript.

REFERENCES

Anderson, M.J. 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.

Balser, T. C. and D. L. Wixon. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology **15**:2935-2949.

Barness, E., Y. Chen, Y. Hadar, H. Marschner, and V. Romheld. 1991. Siderophores of *Pseudomonas putida* as an iron source for dicot and monocot plants. Plant and Soil **130**:231-241.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology **80**:2151-2169.

Bever, J. D., K. M. Westover, and J. Antonovics. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. Journal of Ecology **85**:561-573.

Bontti, E. E., I. C. Burke, and W. K. Lauenroth. 2011. Nitrogen partitioning between microbes and plants in the shortgrass steppe. Plant and Soil **342**:445-457.

Bradford, M. A., N. Fierer, and J. F. Reynolds. 2008. Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen, and phosphorus inputs to soils. Functional Ecology **22**:964-974.

Bru, D., A. Ramette, N. P. A. Saby, S. Dequiedt, L. Ranjard, C. Jolivet, D. Arrouays, and L. Philippot. 2011. Determinants of the distribution of nitrogen-cycling microbial communities at the landscape scale. ISME Journal **5**:532-542.

Burton, J. I., D. J. Mladenoff, M. K. Clayton, and J. A. Forrester. 2011. The roles of environmental filtering and colonization in the fine-scale spatial patterning of ground-layer plant communities in north temperate deciduous forests. Journal of Ecology **99**:764-776.

Castenson, K. L. and M. C. Rabenhorst. 2006. Indicator of reduction in soil (IRIS): Evaluation of a new approach for assessing reduced conditions in soil. Soil Science Society of America Journal **70**:1222-1226.

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology **12**:3137-3149.

Dijkstra, F. A., J. A. Morgan, D. Blumenthal, and R. F. Follett. 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology & Biochemistry **42**:1073-1082.

Drenovsky, R. E., K. L. Steenwerth, L. E. Jackson, and K. M. Scow. 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Global Ecology and Biogeography **19**:27-39.

Enwall, K., I. N. Throback, M. Stenberg, M. Soderstrom, and S. Hallin. 2010. Soil resources Influence spatial patterns of denitrifying communities at scales compatible with land management. Applied and Environmental Microbiology **76**:2243-2250.

Fattorini, M. and S. Halle. 2004. How do filtering effects change in assembling communities after disturbance? . Pages 96-114 *in* V. M. Temperton, R. J. Hobbs, T. Nuttle, and S. Halle, editors. Assembly rules and restoration ecology: Bridging the gap between theory and practice. Island Press, Washington, D.C.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

Fraterrigo, J. M. and J. A. Rusak. 2008. Disturbance-driven changes in the variability of ecological patterns and processes. Ecology Letters **11**:756-770.

Gemborys, S. R. and E. J. Hodgkins. 1970. Forests of small stream bottoms in coastal plain of southwestern Alambama. Ecology **52**:70-&.

Getzin, S., T. Wiegand, K. Wiegand, and F. L. He. 2008. Heterogeneity influences spatial patterns and demographics in forest stands. Journal of Ecology **96**:807-820.

Harper, J. L. 1977. Population biology of plants. Academic Press, London.

Harpole, W. S. and K. N. Suding. 2011. A test of the niche dimension hypothesis in an arid annual grassland. Oecologia **166**:197-205.

Hartman, W. H., C. J. Richardson, R. Vilgalys, and G. L. Bruland. 2008. Environmental and anthropogenic controls over bacterial communities in wetland soils. Proceedings of the National Academy of Sciences of the United States of America **105**:17842-17847.

Hobbs, R. J. and D. A. Norton. 2004. Ecological filters, thresholds, and gradients in resistance to ecosystem reassembly. Pages 72-95 *in* V. M. Temperton, R. J. Hobbs, T. Nuttle, and S. Halle, editors. Assembly rules and restoration ecology: Bridging the gap between theory and practice. Island Press, Washington, D.C.

Holyoak, M., M. A. Leibold, and R. D. Holt. 2005. Metacommunities: Spatial dynamics and ecological communities. The University of Chicago Press, Chicago.

Hooper, D. U., D. E. Bignell, V. K. Brown, L. Brussaard, J. M. Dangerfield, D. H. Wall, D. A. Wardle, D. C. Coleman, K. E. Giller, P. Lavelle, W. H. Van der Putten, P. C. De Ruiter, J. Rusek, W. L. Silver, J. M. Tiedje, and V. Wolters. 2000. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. Bioscience **50**:1049-1061.

Horner-Devine, M. C., J. M. Silver, M. A. Leibold, B. J. M. Bohannan, R. K. Colwell, J. A. Fuhrman, J. L. Green, C. R. Kuske, J. B. H. Martiny, G. Muyzer, L. Ovreas, A. L. Reysenbach, and V. H. Smith. 2007. A comparison of taxon co-occurrence patterns for macro- and microorganisms. Ecology **88**:1345-1353.

Illinois State Geological Survey. Illinois County Boundaries. Second edition. Champaign, Illinois: Illinois State Geological Survey, 2003. [http://www.isgs.uiuc.edu/nsdihome/webdocs/st-basem.html, accessed 25 April 2011]

Jenkinson, B. J. and D. P. Franzmeier. 2006. Development and evaluation of iron-coated tubes that indicate reduction in soils. Soil Science Society of America Journal **70**:183-191.

Jones, C. M. and S. Hallin. 2010. Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. ISME Journal **4**:633-641.

Kardol, P., M. A. Cregger, C. E. Campany, and A. T. Classen. 2010. Soil ecosystem functioning under climate change: Plant species and community effects. Ecology **91**:767-781.

Keddy, P. A. 1992. Assembly and response rules - Two goals for predictive community ecology. Journal of Vegetation Science **3**:157-164.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Kent, A. D., A. C. Yannarell, J. A. Rusak, E. W. Triplett, and K. D. McMahon. 2007. Synchrony in aquatic microbial community dynamics. ISME Journal **1**:38-47.

Lane, D. J. 1991. 16S/23S rRNA sequencing. Wiley and Sons, Chichester, United Kingdom.

LeCoeur, D., J. Baudry, and F. Burel. 1997. Field margins plant assemblages: Variation partitioning between local and landscape factors. Landscape and Urban Planning **37**:57-71.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. Elsevier Science, Amsterdam.

Liu, W. T., T. L. Marsh, H. Cheng, and L. J. Forney. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. Applied and Environmental Microbiology **63**:4516-4522.

Locky, D. A. and S. E. Bayley. 2010. Plant diversity in wooded moderate-rich fens across boreal western Canada: an ecoregional perspective. Biodiversity and Conservation **19**:3525-3543.

Lugtenberg, B. and F. Kamilova. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology **63**:541–545.

Luman, D., T. Tweddale, B. Bahnsen, and P. Willis. 2004. Illinois Map 12, scale 1:500,000-Illinois land cover. Illinois State Geological Survey, Champaign, IL.

Madigan, M. T. and J. M. Martinko. 2006. Brock Biology of Microorganisms. 11th edition. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Matthews, J. W. 2008. Restoration progress and plant community development in compensatory mitigation wetlands. University of Illinois, Urbana-Champaign.

Matthews, J. W., A. L. Peralta, D. N. Flanagan, P. M. Baldwin, A. Soni, A. D. Kent, and A. G. Endress. 2009. Relative influence of landscape vs. local factors on plant community assembly in restored wetlands. Ecological Applications **19**:2108-2123.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology **82**:290-297.

Mentzer, J. L., R. M. Goodman, and T. C. Balser. 2006. Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. Plant and Soil **284**:85-100.

Mitchell, R. J., A. J. Hester, C. D. Campbell, S. J. Chapman, C. M. Cameron, R. L. Hewison, and J. M. Potts. 2010. Is vegetation composition or soil chemistry the best predictor of the soil microbial community? Plant and Soil **333**:417-430.

Mitsch, W. J. and J. G. Gosselink. 2007. Wetlands. Fourth edition. John Wiley and Sons, Hoboken.

Naeem, S., F. S. Chapin III, R. Costanza, P. R. Ehrlich, F. B. Golley, D. U. Hooper, J. H. Lawton, R. V. O'Neill, H. A. Mooney, O. E. Sala, A. J. Systad, and D. Tilman. 1999. Biodiversity and ecosystem functioning: maintaining natural life support processes. Issues in Ecology **4**:11.

National Atlas of the United States. *State Boundaries of the United States* [computer file]. Reston, VA: National Atlas of the United States, 2005. [http://nationalatlas.gov/atlasftp.html , accessed 25 April 2011]

National Atlas of the United States. *Streams and Waterbodies of the United States* [computer file]. Reston, VA: National Atlas of the United States, 2006. [http://nationalatlas.gov/atlasftp.html , accessed 25 April 2011]

Okansen, J., F.G. Blanchet, R. Kindt, P. Legendre, R.B. O'Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, H. Wagner. 2010. The Community Ecology Package. Version 1.17-2.

Paterson, E., A. J. Midwood, and P. Millard. 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. New Phytologist **184**:19-33.

Pett-Ridge, J. and M. K. Firestone. 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Applied and Environmental Microbiology **71**:6998-7007.

Picek, T., M. Simek, and H. Santruckova. 2000. Microbial responses to fluctuation of soil aeration status and redox conditions. Biology And Fertility Of Soils **31**:315-322.

Poll, C., J. Ingwersen, M. Stemmer, M. H. Gerzabek, and E. Kandeler. 2006. Mechanisms of solute transport affect small-scale abundance and function of soil microorganisms in the detritusphere. European Journal of Soil Science **57**:583-595.

Reed, S. C., A. R. Townsend, C. C. Cleveland, and D. R. Nemergut. 2010. Microbial community shifts influence patterns in tropical forest nitrogen fixation. Oecologia **164**:521-531.

Rees, G. N., D. S. Baldwin, G. O. Watson, S. Perryman, and D. L. Nielsen. 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **86**:339-347.

Reynolds, H. L., A. Packer, J. D. Bever, and K. Clay. 2003. Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. Ecology **84**:2281-2291.

Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal **62**:473-480.

Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology **69**:5974-5982.

Sambrook, J. and D. Russell. 2001. Molecular cloning: A laboratory manual.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology **88**:1386-1394.

Schimel, J. P. and J. Bennett. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology **85**:591-602.

Sims, G. K., T. R. Ellsworth, and R. L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Communications in Soil Science and Plant Analysis **26**:303-316.

Sommer, U. 1991. Convergent succession of phytoplankton in microcosms with different inoculum species composition. Oecologia **87**:171-179.

Stephan, A., A. H. Meyer, and B. Schmid. 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. Journal of Ecology **88**:988-998.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Thomashow, L. S. and D. M. Weller. 1990. Role of antibiotics and siderophores in biocontrol of take-all disease of wheat. Plant and Soil **129**:93-99.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham, and F. A. Johnson. 1986. Green, bluegreen and diatom algae - taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie **106**:473-485.

Truu, M., J. Juhanson, and J. Truu. 2009. Microbial biomass, activity and community composition in constructed wetlands. Science of the Total Environment **407**:3958-3971.

Weiher, E. and P. A. Keddy. 1995. The assembly of experimental wetland plant communities. Oikos **73**:323-355.

Wright, J. P., A. S. Flecker, and C. G. Jones. 2003. Local vs. landscape controls on plant species richness in beaver meadows. Ecology **84**:3162-3173.

Yannarell, A. C. and E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology **71**:227-239.

Zobel, M. 1992. Plant species coexistence—the role of historical, evolutionary and ecological filter. Oikos **65**:314-320.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews **61**:533-616.

TABLES

Table 2.1. Summary of predictor variables collected and included in PERMANOVA models.

Spatial scale	Variable (abbreviation)	Included in model
Landscape	precipitation (precip)	YES
	growing degree-days (GDD)	YES
	temperature (temp)	NO
	wetland - surrounding land use (wetland)	YES
	developed land - surrounding land use (developed)	YES
	agriculture - surrounding land use (agriculture)	YES
	barren land - surrounding land use	NO
	upland forest - surrounding land use	NO
	unclassified - surrounding land use	NO
Local soil	ammonium-N	YES
	nitrate-N	YES
	pH	YES
	total organic carbon (TOC)	YES
	total nitrogen (TN)	NO
	soil C:N ratio (C:N ratio)	YES
	redox status	YES
	moisture	YES
Local vegetation type	aquatic	YES
	prairie	YES
	open habitat (open)	YES
	wet meadow	YES
	upland forest (forest)	YES
	mudflat	YES
	emergent wetland (emergent)	YES

Table 2.2. Summary of permutational MANOVA (PERMANOVA) results showing the contribution of landscape effects at the wetland-level (a) and local effects at the wetland (b) and plot-level (c) on plant community variation. Effects were considered significant to contributing to community variation at $P < 0.05$.

(a) Landscape (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
precip	1	0.4474	1.2192	0.0399	0.2018
GDD	1	0.6451	1.7580	0.0575	**0.0120**
wetland	1	0.4536	1.2361	0.0404	0.1548
developed	1	0.7009	1.9100	0.0624	**0.0030**
agriculture	1	0.4975	1.3558	0.0443	0.1079
residuals	21	7.7064		0.6864	
total	26	11.2268		1.0000	

(b) Soil (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	0.6256	1.6383	0.0557	**0.0180**
nitrate	1	0.6318	1.6546	0.0563	**0.0180**
pH	1	0.4068	1.0654	0.0362	0.3536
TOC	1	0.4698	1.2302	0.0418	0.1978
C:N ratio	1	0.3376	0.8842	0.0301	0.6194
redox status	1	0.4565	1.1955	0.0407	0.2078
moisture	1	0.3774	0.9882	0.0336	0.4595
residuals	19	7.2552		0.6462	
total	26	11.2268		1.0000	

(c) Soil (plot-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	1.8180	4.3380	0.0187	**0.0010**
nitrate	1	2.4380	5.8165	0.0251	**0.0040**
pH	1	1.6760	3.9996	0.0172	0.6014
TOC	1	1.1320	2.7013	0.0116	0.2378
C:N ratio	1	0.5650	1.3473	0.0058	0.8082
redox status	1	1.2260	2.9252	0.0126	**0.0140**
moisture	1	1.1010	2.6281	0.0113	0.1548
residuals	203	85.0730		0.8745	
total	210	97.2850		1.0000	

Abbreviations: precip – precipitation, GDD – growing degree-days, wetland – proportion of wetland surrounding site, developed – proportion of developed land surrounding site, agriculture – proportion of agricultural land surrounding site, TOC – total organic carbon, C:N ratio – soil carbon: soil nitrogen (wt/wt).

Table 2.3. Summary of permutational MANOVA (PERMANOVA) results showing the contribution of landscape effects at the wetland-level (a) and local effects at the wetland (b) and plot-level (c) on bacterial community variation. Effects were considered significant to contributing to community variation at $P < 0.05$.

(a) Landscape (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
precip	1	0.1817	1.6657	0.0542	**0.0430**
GDD	1	0.2325	2.1318	0.0694	**0.0100**
wetland	1	0.0921	0.8443	0.0275	0.6434
developed	1	0.0957	0.8774	0.0286	0.5495
agriculture	1	0.0678	0.6219	0.0203	0.9141
residuals	21	2.2902		0.6837	
total	26	3.3497		1.0000	

(b) Soil (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	0.1232	1.4006	0.0368	0.1419
nitrate	1	0.1023	1.1629	0.0306	0.2797
pH	1	0.3628	4.1228	0.1083	**0.0010**
TOC	1	0.1252	1.4224	0.0374	0.1199
C:N ratio	1	0.1551	1.7624	0.0463	**0.0350**
redox status	1	0.1576	1.7910	0.0471	**0.0280**
moisture	1	0.1292	1.4687	0.0386	0.0919
residuals	19	1.6719		0.4991	
total	26	3.3497		1.0000	

(c) Soil (plot-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	0.6030	3.2837	0.0126	**0.0020**
nitrate	1	0.4910	2.6747	0.0103	0.2358
pH	1	3.5090	19.1096	0.0732	**0.0010**
TOC	1	0.8170	4.4520	0.0171	**0.0010**
C:N ratio	1	0.6050	3.2952	0.0126	0.0819
redox status	1	0.9630	5.2467	0.0201	**0.0340**
moisture	1	0.9620	5.2387	0.0201	**0.0010**
residuals	205	37.6420		0.7853	
total	212	47.9320		1.0000	

Abbreviations: precip – precipitation, GDD – growing degree-days, wetland – proportion of wetland surrounding site, developed – proportion of developed land surrounding site, agriculture – proportion of agricultural land surrounding site, TOC – total organic carbon, C:N ratio – soil carbon: soil nitrogen (wt/wt).

Table 2.4. Summary of permutational MANOVA (PERMANOVA) results showing the contribution of landscape effects at the wetland-level (a) and local effects at the wetland (b) and plot-level (c) on denitrifier community variation. Effects were considered significant to contributing to community variation at $P < 0.05$.

(a) Landscape (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
precip	1	0.2855	1.2180	0.0419	0.2168
GDD	1	0.3750	1.5998	0.0550	0.0569
wetland	1	0.2050	0.8746	0.0301	0.6054
developed	1	0.1541	0.6573	0.0226	0.8661
agriculture	1	0.1358	0.5795	0.0199	0.9600
residuals	21	4.9226		0.7226	
total	26	6.8128		1.0000	

(b) Soil (wetland-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	0.1403	0.6341	0.0206	0.8641
nitrate	1	0.1662	0.7513	0.0244	0.7852
pH	1	0.4806	2.1726	0.0705	**0.0030**
TOC	1	0.3686	1.6666	0.0541	**0.0390**
C:N ratio	1	0.2307	1.0428	0.0339	0.3926
redox status	1	0.1816	0.8211	0.0267	0.6693
moisture	1	0.2136	0.9656	0.0314	0.5005
residuals	19	4.2026		0.6169	
total	26	6.8128		1.0000	

(c) Soil (plot-level)

Effect	df	Sums Sqs	*F*-Model	R^2	*P*-value
ammonium	1	0.5500	1.8943	0.0088	**0.0320**
nitrate	1	0.2490	0.8566	0.0040	0.9990
pH	1	2.9010	9.9828	0.0465	**0.0010**
TOC	1	1.6240	5.5871	0.0260	**0.0010**
C:N ratio	1	1.1560	3.9773	0.0185	**0.0170**
redox status	1	0.8860	3.0474	0.0142	0.3237
moisture	1	1.7350	5.9718	0.0278	**0.0010**
residuals	176	51.1420		0.8191	
total	183	62.4380		1.0000	

Abbreviations: precip – precipitation, GDD – growing degree-days, wetland – proportion of wetland surrounding site, developed – proportion of developed land surrounding site, agriculture – proportion of agricultural land surrounding site, TOC – total organic carbon, C:N ratio – soil carbon: soil nitrogen (wt/wt).

Table 2.5. Summary of Mantel test statistic used to correlate dissimilarity matrices. Correlations between communities – landscape factors (a), communities – soil factors (b) at the wetland and plot-levels, and communities –communities (c) at the wetland and plot-levels were based on Pearson's product-moment correlations. Community matrices were based on Bray-Curtis dissimilarity and environmental matrices were based on Euclidean distances.

(a) Community – landscape comparison

	Wetland-level
Comparison	**Pearson's correlation**
bacteria – landscape	$r_M = 0.2860$, $P = 0.009$
denitrifier – landscape	$r_M = 0.1932$, $P = 0.050$
plant – landscape	$r_M = 0.2887$, $P = 0.001$

(b) Community – soil comparison

	Wetland-level	*Plot-level*
Comparison	**Pearson's correlation**	**Pearson's correlation**
bacteria – soil	$r_M = 0.6461$, $P = 0.001$	$r_M = 0.4706$, $P = 0.001$
denitrifier – soil	$r_M = 0.2467$, $P = 0.036$	$r_M = 0.2513$, $P = 0.001$
plant – soil	$r_M = 0.2384$, $P = 0.003$	$r_M = 0.1312$, $P = 0.001$

(c) Community comparison

	Wetland-level	*Plot-level*
Comparison	**Pearson's correlation**	**Pearson's correlation**
bacteria – plant	$r_M = 0.3258$, $P = 0.001$	$r_M = 0.2162$, $P = 0.001$
denitrifier - plant	$r_M = 0.3048$, $P = 0.001$	$r_M = 0.2158$, $P = 0.001$

FIGURES

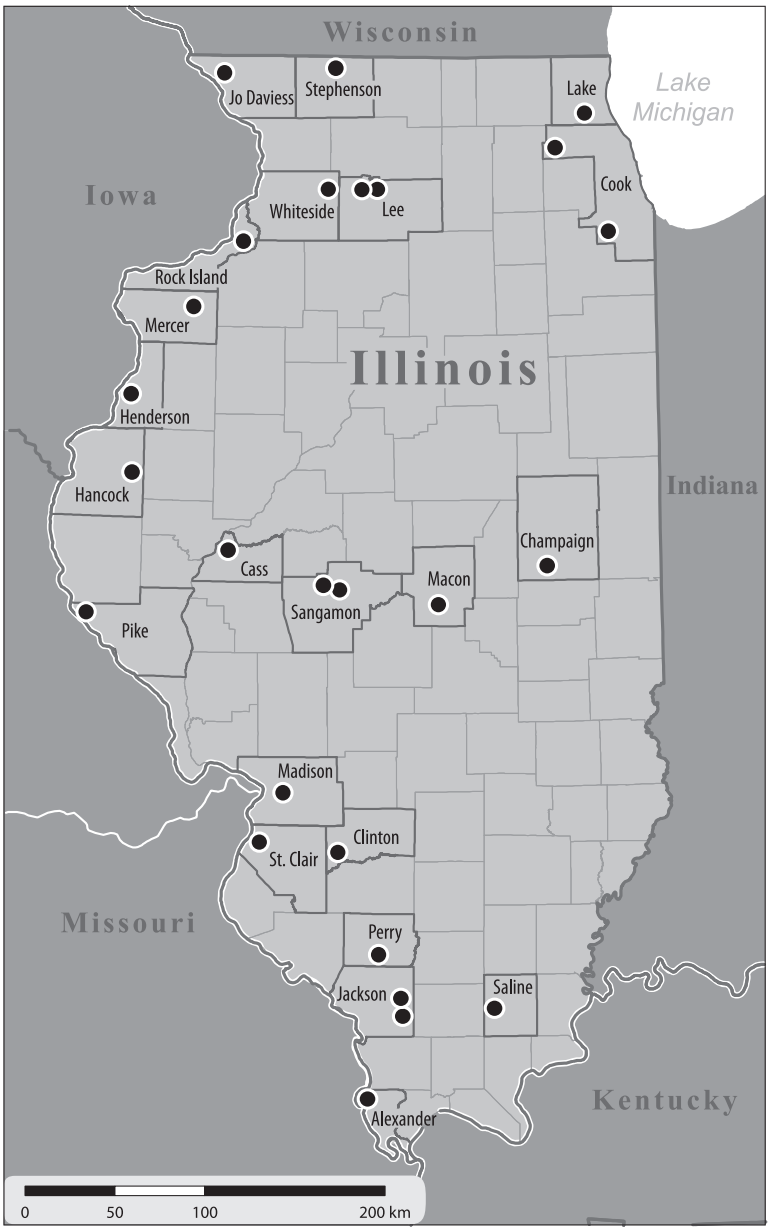


Figure 2.1. Illinois map showing sampling points of restored wetlands sampled in this study. Counties names are included where wetlands were sampled. Map was produced by R.R. Lash. Map data came from the following sources (National Atlas of the United States 2005, 2006; Illinois State Geological Survey, 2003). The map data were compiled using ArcGIS 9.3 and the map was produced using Adobe Illustrator CS4.

CHAPTER 3

DISTRIBUTION OF DENITRIFYING AND AMMONIA OXIDIZING MICROORGANISMS ALONG A MOISTURE GRADIENT

ABSTRACT

Gradients in abiotic parameters, such as soil moisture, can strongly influence microbial structure and function. Denitrifying and ammonia oxidizing microorganisms, in particular, have contrasting physiological responses to abiotic factors such as oxygen concentration and soil moisture. Identifying which abiotic factors govern the composition and activity of denitrifying and ammonia oxidizing microorganisms is critical for understanding nitrogen cycling functions. The objectives of this study were to (i) examine denitrifier and archaeal ammonia oxidizer community composition and (ii) assess taxa occurrences within each functional group related to soil conditions along an environmental gradient. Soil was sampled across four transects at four locations along a dry to saturated environmental gradient at a restored wetland. Soil pH and soil organic matter content increased from dry to saturated plots. Composition of soil denitrifier and ammonia oxidizer functional groups was assessed by terminal restriction length polymorphism (T-RFLP) community analysis, and local soil factors were also characterized. Microbial community composition differed along the gradient due to variable taxon-level specific responses to soil moisture (denitrifier: ANOSIM R = 0.739, $P < 0.001$; ammonia oxidizers: ANOSIM R = 0.760, $P < 0.001$). Individual denitrifier taxa were observed over a larger range of moisture levels than individual archaeal ammonia oxidizer taxa. Together, our data suggest that variation in environmental tolerance of microbial taxa can shape the ecosystem-level responses influencing nitrogen cycling in terrestrial ecosystems.

INTRODUCTION

Microorganisms capable of tolerating a range of environmental conditions are commonly observed in soil environments. The influence of the local environment can mediate changes in community composition and function (Dumbrell et al. 2010, Gutknecht et al. 2006, Ikenaga et al. 2010, Pett-Ridge and Firestone 2005, Rousk et al. 2010, Swan et al. 2010). An organism's growth and survival occur within a specific set of abiotic parameters (e.g., temperature, pH, redox potential) (Shelford 1913). Survival in a heterogeneous environment requires that an organism be able to tolerate the range of physical and chemical factors presented by its local environment. Thus, variations in habitat specialization among microbial taxa can result in a narrow or wide range in environmental tolerance, where taxon-level responses can contribute to microbial community-level changes across environmental gradients.

Habitat specialization is an attribute of a species and its response to a range of abiotic parameters in its local environment (Grinnell 1917, Hughes 2000, Silvertown et al. 2006, Soberon 2007). Microbes that carry out facultative metabolic pathways can have more generalized habitat preferences than microbes that carry out obligate metabolic pathways, which may be more restricted in their distribution (Giri et al. 2005). For example, facultative anaerobic microbes can live in the presence or absence of oxygen and are capable of utilizing one or more alternative terminal electron acceptors, whereas obligate aerobic microbes are incapable of anaerobic metabolism. As microorganisms respond to their local environment differently, we can expect ecosystem-level changes in microbial functions as well (Strickland et al. 2009).

Oxygen availability in soils and sediments fluctuates with changes in water levels, significantly influencing microbial metabolism that is directly linked to nutrient cycling processes (DeAngelis et al. 2010, Gutknecht et al. 2006, Pett-Ridge and Firestone 2005, Picek et

al. 2000). Dynamic wetland hydrology can result in recharge of oxygen and nutrient pools, whereas less dynamic hydrology can result in a more stable, potentially more nutrient-limited environment where oxic or anoxic conditions persist (Keddy 2000). As a consequence, fluctuating hydrology provides diverse wetland habitats for microorganisms. Under dynamic hydrology, microbial communities should comprise taxa with the capacity to tolerate a range of soil moisture and redox conditions, whereas stable habitats would be expected to harbor taxa with a narrow range of tolerances (DeAngelis et al. 2010, Pett-Ridge and Firestone 2005, Picek et al. 2000). With greater understanding of proximal roles of the environment on the microorganisms that participate in critical nutrient cycling processes, we can improve our ability to predict microbially-mediated ecosystem functions in response to fluctuations in conditions brought about by environmental change.

Transformations in the nitrogen (N) cycle are carried out by microbial taxa with a range of physiological tolerances. These taxa carry out transformations that alter the mobility of nitrogen in the environment, and can to lead to the removal or retention of nitrogen species in ecosystems (Francis et al. 2007, Wallenstein et al. 2006). Nitrate (NO_3^-) removal occurs via the denitrification pathway ($NO_3^- \rightarrow N_2$), while nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) is one mechanism for internal N cycling (Francis et al. 2007, Wallenstein et al. 2006). Microbially mediated N transformations such as denitrification and nitrification are particularly sensitive to abiotic influences such as oxygen concentration (Bodelier et al. 1996, Jayakumar et al. 2009). Microbial taxa within these N-specific functional groups are also phylogenetically distinct. For example, denitrifiers belong to a phylogenetically broad group of facultative anaerobes that occupy a variety of environments (Nogales et al. 2002, Rich et al. 2003, Rich and Myrold 2004, Wallenstein et al. 2006). Because of their facultative metabolism, denitrifiers, as a functional

group, are physiologically tolerant and have been characterized as having cosmopolitan distributions and broad habitat (Nogales et al. 2002, Peralta et al. 2010, Rich et al. 2003, Rich and Myrold 2004, Wallenstein et al. 2006). Specifically, denitrifiers carrying the *nosZ* gene are broadly distributed among habitat types in agricultural, forest, meadow, and successional fields (Cavigelli and Robertson 2000, Rich et al. 2003, Wallenstein et al. 2006). Denitrification has been documented to range to occur at varying pH from acidic to alkaline conditions (reviewed by Simek and Cooper 2002). A previous study demonstrated that *nosZ* sequences from forest or meadow soils were not phylogenetically distinct, indicating a lack of habitat specialization (Rich et al. 2003).

In contrast to denitrifiers, the ammonia oxidizers that carry out the first step in nitrification are obligate aerobes and are restricted within the *Betaproteobacteria* and mesophilic *Crenarchaeota* (Francis et al. 2007, Kowalchuk and Stephen 2001). In particular, archaeal ammonia oxidizers, although globally distributed, are locally specialized by habitat. Evidence based on *amoA* gene sequences, used as a molecular marker of ammonia oxidizing bacteria and archaea, has revealed that ammonia oxidizer distribution is dominated by distinct lineages in different habitats (e.g., marine sediments, estuarine sediments, soil) (Erguder et al. 2009, Francis et al. 2007, Francis et al. 2005, Prosser and Nicol 2008). Particular archaeal *amoA* ecotypes may be constrained to a narrow range of conditions and may display rapid community turnover along environmental gradients (Caffrey et al. 2007, Erguder et al. 2009, Francis et al. 2007, Francis et al. 2005, Martens-Habbena et al. 2009, Moin et al. 2009, Prosser and Nicol 2008). Because of their contrasting physiologies, denitrifier and ammonia oxidizer functional groups are ideal communities for examination of the relationship between habitat specialization and community composition.

In Chapter 2 of this dissertation, the survey of total bacterial and denitrifier community composition among restored wetlands, precipitation at the landscape-level and moisture and soil nutrients at the local plot-level significantly contributed to bacterial variability. Redox condition and nutrient levels also influenced denitrifier community composition. To further explore the relationship between soil fertility and moisture and microbial communities within a wetland, we assessed microbial community composition associated with specific nitrogen cycling transformations along an environmental gradient. The objectives of this study were to (i) examine denitrifier and ammonia oxidizer community composition and (ii) assess taxa occurrences (based on terminal restriction fragment (T-RF) discrimination of different taxa) of denitrifiers and ammonia oxidizers along an environmental gradient surrounding a floodplain lake. We predicted that denitrifier and ammonia oxidizer community composition would vary along the moisture gradient. Denitrifying bacteria were expected to be a broadly distributed functional group composed of both habitat specialist taxa that occupy specific locations along the gradient and habitat generalist taxa found across the gradient (Fig. 3.1a). Ammonia oxidizer taxa were expected to be locally restricted along the entire gradient (Fig. 3.1b). We aim to address ecological mechanisms underlying varying responses of N cycling microorganisms to environmental heterogeneity associated with changes in soil moisture.

MATERIALS AND METHODS

Field sampling

The study was carried out at Emiquon Preserve in Lewistown, Illinois (40.3584 N, 90.085 W). Emiquon Preserve contains approximately 3000 hectares of land bordering the Illinois River. In 2007, restoration from row crop conventional agriculture to floodplain wetland

began at Emiquon Preserve. Artificial drainage of the area ceased in 2007, allowing precipitation to refill Thompson Lake.

Samples were collected monthly from June until September of 2008. Four replicate transects (85 m apart) were established perpendicular to the Thompson Lake shoreline along an increasing moisture gradient from upland prairie to saturated floodplain wetland along 1-m elevation contours (elevation differed by 1-m between adjacent plots) (Fig. 3.2). Four 4-m^2 plots (A-D) were placed along each transect. Elevation was used as a proxy for water level. In each plot, samples were collected within a 1-m^2 quadrat. Plant species cover class was assigned (<1%, 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, or 96-100%) and recorded within each quadrat to survey herbaceous vegetation. Emergent vegetation was not observed in the constantly submerged plots (D).

Six soil samples (12 cm depth) were collected within each plot (A-D) using a soil corer (3 cm diameter) and combined into a single composite sample representative of each discrete moisture condition (each plot) for all four replicate transects along the upland to wetland gradient. The soil depth chosen represented an environment influenced by environmental and plant inputs. In the field, soil samples were kept on ice and transported back to the laboratory for processing and storage. Soils for molecular microbial analyses were frozen at -20 °C prior to DNA extraction.

Soil analyses

For all samples, gravimetric soil moisture was determined from a 20-30 g subsample from each plot for each sampling date. Field moist soil was dried at 105 ºC for 24 hours and moisture content was determined from the proportion of water (by weight) to oven-dried soil. In addition, a composite sample from each plot for each sampling date was analyzed for soil

texture. Texture analysis was performed on a 50 g sample using the hydrometer method (Gee and Bauder 1979). Organic matter analysis and pH were analyzed on soil samples collected in June. A subsample of air-dried soil was analyzed to determine total organic matter (% total C and total N, % organic matter). Elemental analysis of C and N on duplicate soil samples was completed using combustion methods (LECO TrueSpec™ analyzer (LECO Corp., St. Joseph, MI) (Wang and Anderson 1998). Soil pH was determined for a 5 g sample analyzed using a 1:1 soil:water ratio on duplicate samples and the average pH was reported.

DNA extraction and purification

Samples were freeze-dried prior to total genomic DNA extraction using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH). Genomic DNA was purified using a cetyl trimethyl ammonium bromide (CTAB) extraction to remove contaminating humic acids (Sambrook and Russell 2001). Soil DNA concentration was adjusted to a standard concentration of 10 ng μl^{-1}.

Microbial community analyses

Composition of denitrifying microorganisms was assessed using terminal restriction fragment length polymorphism (T-RFLP). In this study, "denitrifier taxa" refers specifically to the subset of microorganisms that carry out the last step of denitrification ($N_2O \rightarrow N_2$) catalyzed by nitrous oxide reductase. PCR reactions to amplify the *nosZ* gene, which encodes the catalytic subunit of nitrous oxide reductase, contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of *Taq* polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 µl. The *nosZ* gene was amplified using *nosZ*-F-1181, 5'-CGCTGTTCITCGACAGYCAG-3' and *nosZ*-R-1880, 5'-ATGTGCAKIGCRTGGCAGAA-3' to yield a 700 bp PCR product (Rich et al.

2003). The *nosZ* reverse primer was labeled with the phosphoramidite dye 6-FAM. Reactions were cycled with initial denaturation at 94 °C for 3 min, followed by 25 cycles of 94 °C for 45 s, 56 °C for 1 min, and 72 °C for 2 min, with a final extension carried out at 72 °C for 7 min. The Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA) was used to combine and concentrate *nosZ* PCR product from two 50 µl reactions. Amplicons generated from each sample were digested in single-enzyme restriction digests containing *Alu*I and *Hha*I.

Community composition of ammonia oxidizing archaea was assessed using T-RFLP analysis of the *amoA* gene encoding the catalytic α-subunit of archaeal ammonia monooxygenase. This enzyme is responsible for catalyzing the rate-limiting first step of nitrification (Kowalchuk and Stephen 2001). PCR reactions to amplify the *amoA* gene contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of Taq polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 µl. The *amoA* gene was amplified using *amoA-F*, 5'-STAATGGTCTGGCTTAGACG-3' and *amoA-R*, 5'-GCGGCCATCCATCTGTATGT-3' to yield a 650 bp amplicon (Francis et al. 2005). The *amoA* forward primer was labeled with the fluorescent dye HEX, the *amoA* reverse primer was labeled with the fluorescent dye NED. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 53 °C for 1 min, and 72 °C for 1 min, with a final extension carried out at 72 °C for 15 min. The Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA, USA) was used to concentrate *amoA* PCR product from a single 50 µl reaction. PCR products amplified from each sample were digested with *Rsa*I, yielding 2 fluorescently-labeled terminal restriction fragments (T-RFs) from each amplicon. Bacterial *amoA* T-RFLP was not carried out because quantitative PCR results revealed that bacterial *amoA* (AOB) gene abundance was less than 70 copies of the

AOB gene ng^{-1} DNA, whereas archaeal *amoA* (AOA) ranged from about 1,400 to 16,000 copies of the AOA gene ng^{-1} DNA along the environmental gradient.

The length and relative abundance of terminal restriction fragments (T-RFs) were determined by denaturing capillary electrophoresis using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 63 °C and 15 kV with a run time of 120 minutes using POP-7 polymer. The ABI GeneScan ROX 1000 size standard (Applied Biosystems, Foster City, CA) was used as the internal size standard for the *nosZ* and *amoA* T-RFLP analysis. Size-calling was carried out using GeneMarker version 1.85 (SoftGenetics, State College, PA). For our analyses, each terminal restriction fragment represents a microbial taxon, and the combination of all T-RFs produced from a sample corresponds to the assemblage of microorganisms present in a soil sample. In order to account for run-to-run variations in signal detection, each T-RF was expressed as a proportion of the observed community by normalizing the signal strength (e.g. peak area) of each T-RF peak to the total fluorescence observed for each digest (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Terminal restriction fragments that exceeded a detection threshold of 100 relative fluorescence units were included in community analyses. Normalized T-RFLP profiles produced from separate digests of each PCR product (or from different fluors, in the case of *amoA*) were concatenated prior to statistical analysis (Fierer and Schimel 2003, Peralta et al. 2010).

Statistical analyses

Because there was no significant temporal variation in the community composition (data not shown), relative T-RF signal strength for each microbial taxon (each T-RF) was averaged over the four months within each plot, and similarity matrices were generated separately for denitrifer and ammonia oxidizer communities by calculating the Bray-Curtis similarity

coefficient for each pair of samples (Legendre and Legendre 1998). Relative quantification of amplicons assessed by capillary electrophoresis has been shown to be highly correlated to direct quantification based on qPCR (He et al. 2010), so T-RF relative fluorescence data was treated as relative abundance data in our statistical procedures, as is common in microbial ecology studies (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). All statistical analyses were based on the average relative abundance of T-RFs over four time points. Analysis of similarity (ANOSIM) was used to test for significant differences in community composition among locations along the environmental gradient for microbial and plant communities (n = 4 replicate transects). An ANOSIM R statistic was generated based on comparison of rank similarity within and among groups of samples, and significance of the group dissimilarity was based on permutation tests. An R-value of 1 indicates complete dissimilarity among groups and R = 0 indicates a high degree of community similarity among locations along the gradient. Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship among microbial communities along the moisture gradient. Nonmetric multidimensional scaling is based on the rank order relation of dissimilarities based on the Bray-Curtis similarity coefficient, where the largest distance between points on the NMDS ordination represents the most dissimilar microbial communities. To test the relationship between community composition and environmental factors, the BIO-ENV procedure in PRIMER was used. This procedure is based on a Spearman's rank correlation and identifies which environmental variables are correlated to the community patterns. Calculation of similarity coefficients, ANOSIM, NMDS, and BIO-ENV were carried out using PRIMER version 6 (PRIMER-E Ltd, Plymouth, UK).

The similarity percentages routine (SIMPER) carried out in PRIMER 6 was used to evaluate each taxon's contribution to group similarity along the moisture gradient. Similarities

among samples within each moisture condition, designated as a plot, were calculated and taxa contribution to within-group similarity was assessed. Taxa contributing greater than ~5% to within-group similarity were identified (Table B.3).

To visualize taxa occurrence (represented by T-RF) for denitrifiers and ammonia oxidizers along the moisture gradient, the heatmap.2 function in the R Statistics Package (R Development Core Team 2010) was used. Taxa observed in four or more plots were included in the analysis. Hierarchical cluster analysis was used to group taxa according to occurrence along the defined moisture gradient.

Venn diagrams were used as an additional visualization technique to more clearly observe overlap of denitrifiers and ammonia oxidizer taxa along the moisture gradient. Taxa observed in four or more plots were included in the analysis. Venn diagrams were generated (and proportioned according to total number of taxa represented) using the Venn Diagram Plotter developed at Pacific Northwest National Laboratories (http://omics.pnl.gov). A taxon was considered present for the underlying presence-absence matrix if it was observed in at least two of four of the replicate plots within a particular moisture condition.

Range in soil moisture (i.e. "habitat breadth" for each taxon based on this parameter) was calculated as the difference in maximum and minimum soil moisture for each where each denitrifier and ammonia oxidizer T-RF was observed along the gradient. Taxa observed in four or more plots were included in this habitat breadth analysis. The distribution of the habitat breadth for moisture conditions was compared between the functional groups using the non-parametric Wilcoxon rank sum test in the R Statistics Package (R Development Core Team 2010).

RESULTS

Evaluation of the environmental gradient

Along each transect (from upland to submerged plots) (Fig. 3.2), there was an increase in soil moisture, organic matter (%TOC, %TN), pH, and clay content (Table 3.1a). Texture transitioned from coarsely textured soils in the dry, upland plots to finely textured soils in the saturated plots. Texture observed in dry plots (plots A and B in each transect) ranged from sandy clay loam to sandy loam. The transition plots (plot C) were characterized as silty, clay and saturated plots (plot D) were mainly clay in texture.

Plant community composition was significantly different among plots along the environmental gradient (ANOSIM R = 0.833, $P < 0.05$) (Fig. B.1, Table B.1). The plant community was mainly dominated by old field species common to recently abandoned agricultural areas. The driest plots were dominated by *Conyza canadensis* (horseweed) in upland plots (plot A) and also *Ambrosia artemisiifolia* (ragweed) (plot B) (Table B.1). The transition locations (plot C) were dominated by *Conyza canadensis* and the exotic species *Setaria faberi* (giant foxtail) (Table B.1). No emergent vegetation was present in the completely inundated plots (plot D at each transect).

Community composition along a moisture gradient

While a number of chemical and physical parameters varied along the environmental gradient, we were specifically interested in the distribution of ammonia oxidizers and denitrifiers in relation to soil moisture. A total of 178 unique *nosZ* and 80 *amoA* terminal restriction fragments (T-RFs) were detected in the denitrifying and archaeal ammonia oxidizing communities, respectively. Community composition was quite variable in the upland plots, but analysis of similarity could not distinguish the assemblages in the A and B locations on the

sampling transects (ANOSIM R = 0.177, $P > 0.05$) (Fig. 3.3a, Table 3.2a). Denitrifier composition in saturated plots was significantly different than communities found in upland locations (comparison of samples from upland plots (A) and saturated plots (D): ANOSIM R = 0.719, $P < 0.001$) (Fig. 3.3a, Table 3.2a). As observed for denitrifiers, the upland plots (A and B) also harbored similar assemblages of ammonia oxidizers (ANOSIM R = 0.104, $P > 0.05$) (Fig. 3.3b, Table 3.2b). Ammonia oxidizer communities were significantly different between the wet-dry transition zone (C), saturated conditions (D) and upland (A and B) plots (ANOSIM R = 0.760, $P < 0.001$) (Fig. 3.3b, Table 3.2b). For both ammonia oxidizers and denitrifiers, the assemblages observed in the upland plots (A and B) were more variable across all transects than the assemblages of each functional group observed at the more saturated sites (C and D) (Fig. 3.3). Changes in environmental conditions were correlated with patterns in community structure in ammonia oxidizers (Spearman's $\rho = 0.72$, $P = 0.0017$) but not denitrifiers (Spearman's $\rho = -0.12$, $P = 0.6643$) (Fig. B.2). In addition to soil moisture changing along the environmental gradient, other soil factors significantly contributed to the biotic patterns observed in community composition. For example, total nitrogen and pH were most strongly correlated with the patterns in the denitrifier community ($\rho = 0.666$, $P < 0.05$), while pH and percent clay best explained the biotic pattern for archaeal ammonia oxidizers ($\rho = 0.467$, $P < 0.05$). For both functional groups, it appeared that increasing soil moisture (along with other changes along the environmental gradient) functioned as an environmental filter that constrained composition.

Microbial taxa response to moisture gradient

A total of 81 denitrifier taxa met the criteria for inclusion in the heat map and Venn diagrams (Figs. 3.4, 3.5). A group of generalist taxa were detected in plots that spanned the moisture gradient. These generalists included two highly abundant denitrifier taxa (H-225, A-

413) that strongly contributed to within-group similarity at each position along the moisture gradient (Fig. 3.4a, Table B.2) and a core group of taxa with broad distribution but relatively low abundance (e.g., H-213, H-246, A-289, H-113, A-203) was observed along the entire gradient (Fig. 3.4a). In addition, a great deal of overlap in taxa was observed between upland plots (A and B) dry-wet transition (plot C) locations (Fig. 3.5a). The community observed in the saturated sites (plot D) included a subset of taxa that were unique to the saturated conditions (Figs. 3.4a, 3.5a).

A total of 43 ammonia oxidizer taxa met the criteria for inclusion in the heat map and Venn diagrams (Figs. 3.4, 3.5). Ammonia oxidizers, as a functional group, were observed at all plots along the moisture gradient (Fig. 3.4b). Plots A and B shared a large proportion of taxa, but a high turnover in ammonia oxidizers was observed from the dry to transition to saturated plots, in contrast with the denitrifiers, which showed a higher proportion of shared taxa along the gradient (Fig. 3.5). The denitrifiers were, in general, broadly distributed across moisture conditions. In contrast, a subset of ammonia oxidizer taxa was observed to achieve high relative abundance at discrete locations along the gradient (Fig. 3.4b). For example, T-RF Y-431 was consistently observed to be a dominant taxon observed in the ammonia oxidizer communities in the saturated plots, whereas G-572 was detected in high relative abundance in the upland plots. Ammonia oxidizer T-RF Y-571 significantly contributed to community similarity within the wet-dry transition (plot C) locations (Table B.3). In addition, specific T-RF's in relatively low abundance were restricted to specific locations along gradient (e.g., Y-165, G-334, Y-247) (Fig. 3.4b). The distribution of soil moisture tolerance between denitrifiers and ammonia oxidizers was significantly different (Wilcoxon rank sum, W=0.2413, *P*-value = 0.0002) (Fig. B.3).

Average soil moisture range (habitat breadth) was higher for denitrifier taxa than ammonia oxidizers (Fig. B.3).

DISCUSSION

Microbial communities along the moisture gradient

Organisms vary in habitat specificity as a consequence of their tolerance to a suite of physical and chemical conditions within the local environment (Shelford 1913). Thus, taxon-specific responses to the local environment can cause shifts in microbial community composition in space and time (Decaens 2010, Smith et al. 2010, Swan et al. 2010). A well-characterized environmental gradient provided a unique opportunity to compare ecological influences on two microbial functional groups involved in N cycling. The gradient in soil moisture (11.6 to 46.6%) at the floodplain wetland in this study was accompanied by changes in total organic carbon, pH, soil texture and plant community composition, providing a variety of potential habitats for soil microorganisms (Tables 3.1, 3.2C). Despite changes in soil moisture over the sampling period (Table 3.1b), microbial community composition remained constant over time (data not shown).

Changes in pH influence availability of carbon and mineral nitrogen available for denitrifying microorganisms (Simek and Cooper 2002), and different denitrifier communities have also been found to respond to varied carbon sources (Henderson et al. 2010). We found that patterns in both denitrifier and archaeal ammonia oxidizer communities were correlated to changes in soil pH. Our findings, from an unmanipulated restored wetland, corroborate previous studies using an experimental pH gradient (Cavigelli and Robertson 2000, Nicol et al. 2008).

Potential denitrification and nitrification rates measured at the last time point in our time series were highest in the most saturated plots. These saturated plots were also associated with

more alkaline conditions and highest carbon content (Fig. B.4, Table 3.1). In a previous study, total nitrogen gas emissions (N_2O, NO, N_2) were observed to be lower in acidic compared to neutral or slightly alkaline conditions (Simek and Cooper 2002).

Environmental variation commonly contributes to changes in denitrifier and ammonia oxidizer community composition from managed agricultural to unmanaged forest ecosystems and in marine sediments (Bernhard et al. 2010, Caffrey et al. 2007, Francis et al. 2007, Francis et al. 2005, Kowalchuk and Stephen 2001, Martens-Habbena et al. 2009, Moin et al. 2009, Philippot 2002, Prosser and Nicol 2008, Rich et al. 2003, Wallenstein et al. 2006, Zumft 1997). In contrast to previous work, we found that ammonia oxidizer community composition was more strongly influenced by the environmental gradient than the denitrifier community composition This relationship was evidenced by correlated changes between the local soil environment and ammonia oxidizer community structure (Fig. B.2).

Community-level changes induced by differences in taxon-level patterns

Patterns of denitrifier and ammonia oxidizer communities were significantly different along the moisture gradient (Fig. 3.3). In addition, a wider range in habitat breadth was observed for the majority of denitrifier taxa (24-26% moisture range) than for ammonia oxidizer taxa (20-22% moisture range) (Fig. B.3). Distinct patterns in community composition between denitrifiers and ammonia oxidizers were, in part, driven by different taxon-level relationships with the environmental gradient. Dominant dentrifier taxa were the same in all plots along the gradient (Table B.2). Denitrifier T-RFs H-225 and A-413 contributed 13-28% to within-plot similarity at discrete positions along the gradient (Table B.2). In contrast, unique ammonia oxidizer taxa dominated different plots along the gradient (Table B.3). The dominant ammonia oxidizer taxon changed from dry (plot A/B: G-572) to transition and saturated plots (plot C: Y-571; plot D: Y-

431) (Fig. 3.4). A high proportion of denitrifier taxa overlapped between upland and transition plots of the moisture gradient (plots B/C), while ammonia oxidizers shared fewer taxa from dry to saturated plots (Fig. 3.5).

Microbial community composition in the saturated plots was distinct from other communities along the gradient. Ammonia oxidizer taxa exhibited higher turnover than denitrifier taxa in the upland and wet-dry transition plots along the gradient (Fig. 3.5). Taxa had varying contributions to compositional changes along the gradient: dominant ammonia-oxidizing archaea contributed to unique composition in each moisture level, while dominant denitrifiers spanned the range of soil moisture and contributed to similarity among all plots (Fig. 3.4). Because denitrifiers are facultative anaerobes, they are not as sensitive to oxygen limitation as ammonia oxidizers, and this difference in physiology may be a key contributor to the observed patterns (Blom 1999, Bodelier et al. 1996, Bodelier et al. 1997). It is also possible for specific ammonia oxidizer taxa to adapt to microaerophilic environments (Francis et al. 2007, Francis et al. 2005) and persist in saturated soils, which was observed in our study. Particular ammonia oxidizer taxa appear to be adapted to specific ranges of soil moisture in this wetland; a number of unique taxa were observed in each plot, particularly the transition C and saturated D plots (Figs. 3.4 and 3.5). Because environmental conditions are more variable in the transition zones (plot C), microbial taxa must be adapted to the redox fluctuations, so taxa in this location would need to be tolerant of variable conditions. In addition, continuously saturated plots (D) are expected to be particularly stressed for oxygen, and only a subset of ammonia oxidizer taxa (likely microaerophilic specialists) were observed in this habitat.

Habitat generalists vs. specialists along environmental gradients

Ammonia oxidizer taxa are more limited by soil moisture (and redox) conditions than denitrifiers. Ammonia oxidizers are sensitive to pH as it influences substrate availability; consequently, activity is inhibited below about pH 6.5 (Prosser 2007). Denitrification, in contrast, has been documented to be influenced by pH, but can occur over a wide range from about pH 4 to pH 8 and based on their review of the current literature, an optimum pH for denitrification was not obvious (Simek and Cooper 2002). Both functional groups are also sensitive to substrate availability, where low levels of nitrate and ammonium support lower rates of denitrification and nitrification, respectively (Prosser 2007, Zumft 1997). Taxa within the ammonia oxidizer functional group are characterized by narrow habitat breadth. In contrast, individual denitrifier taxa exhibit wider tolerance of soil moisture conditions, and consequently, a wider habitat breadth. Taxa with wider habitat breadth are expected to display a higher degree of taxon overlap (or co-occurrence of taxa) along environmental gradients. Generalist taxa are characterized by having a wide environmental tolerance compared to specialist taxa, which are restricted to a subset of environmental conditions. These differences in habitat specificity can contribute to nested environmental tolerance, where a large number of taxa co-occur under a particular set of abiotic parameters. As environmental conditions get more extreme (e.g., very dry, saturated), some taxa are unable to persist and only a subset of the original community remains (Worthen 1996). We observed that the denitrifiers exhibited a more nested structure, where upland communities (plots A and B) consisted of subsets of the communities observed in the wet-dry transitional part of the gradient (Fig. 3.5a). In contrast, ammonia oxidizers displayed a greater turnover in taxa as a result of spatial heterogeneity from upland to transition plots, and a weaker pattern of nestedness compared to denitrifiers (Fig. 3.5b). For both functional groups,

communities of the most saturated plots were very different from those of the upland and transition plots (Figs. 3.3 & 3.4). Understanding the potential for nested subset structure to develop under particular conditions as observed in the present study, can provide information needed to predict community structure in restored wetlands.

Potential for functional redundancy

Describing a community in terms of "response functional traits" rather than phylogenetically-based approaches can provide information on how communities responsible for specific ecosystem functions respond to environmental perturbation (Naeem and Wright 2003, Petchey and Gaston 2006). The biological insurance hypothesis (Botton et al. 2006, Folke et al. 1996, Loreau et al. 2003, Naeem and Li 1997) posits that greater biodiversity, resulting in a higher potential for functionally redundant species to assemble, can increase ecosystem stability and provide a buffer against fluctuations or disruption of microbial functions (Botton et al. 2006). We show that the "insurance value" of biodiversity varies between denitrifier and ammonia oxidizer functional groups. For example, at the taxon-level, changes in soil moisture can influence narrowly-distributed ammonia oxidizers more than broadly-distributed denitrifiers, resulting in a more drastic change in ammonia oxidizers communities during a unique flooding or drying event. This scenario was actually observed in the present study, where ammonia oxidizers in the transition C plots varied significantly in community composition compared to communities at different locations along the moisture gradient (Figs. 3.3b & 3.4b). If hydrologic conditions were altered from current patterns, then particular ammonia oxidizer populations would no longer be able to persist under drier or wetter conditions. This scenario would result in a shift in ammonia oxidizers towards taxa that are more tolerant of the new environmental conditions, while existing denitrifiers may be able to tolerate hydrological alterations. It is also

possible that a changing in drying-flooding conditions may result in a decrease in number of taxa and a decrease in potential functional redundancy. In the present study, temporal variability in moisture did not influence community composition, so the particular range in environmental factors experienced along gradient is more important to microbial community composition than short-term changes. Our findings highlight the importance of functional redundancy in the relationship between community structure and function. We suggest that experimental studies of resistance to disturbance in the context of functional redundancy will contribute significantly to our understanding of overall wetland function. We have shown that abiotic factors do not have equivalent effects on all microorganisms. It will be particularly important to continue to improve our understanding of how taxa within N-specific functional groups vary in tolerance to a suite of abiotic factors. Such an understanding will enable more accurate prediction of community structure and potential function of N cycling transformations with respect to local environmental conditions.

ACKNOWLEDGEMENTS

This chapter was completed in collaboration with Dr. Jeffrey Matthews and Dr. Angela Kent. Emiquon Preserve is being restored by The Nature Conservancy. We would like to thank M. Lemke, S. McClure, T. Hobson, and D. Blodgett for logistical assistance in the field. S. Paver, D. Nelson, J. Tsai, and R. Darmody provided technical assistance in the laboratory and R. Lankau and E. Wheeler for statistical assistance. S. Paver, O. Sinno and R. Andrus assisted in the field. Y. Cao, J. Dalling, M. Wander, K. Amato, S. Paver, C. Allsup, D. Keymer, E. Wheeler, C. Balakrishnan, and A. Yannarell contributed helpful comments to earlier versions of this manuscript. This work was supported by the Cooperative State Research, Education and

Extension Service, U.S. Department of Agriculture, under project number ILLU 875-374. This research was also supported, in part, by the Program in Ecology, Evolution, and Conservation Biology at the University of Illinois at Urbana-Champaign

REFERENCES

Bernhard, A. E., Z. C. Landry, A. Blevins, J. R. de la Torre, A. E. Giblin, and D. A. Stahl. 2010. Abundance of ammonia-oxidizing archaea and bacteria along an estuarine salinity gradient in relation to potential nitrification rates. Applied and Environmental Microbiology 76:1285-1289.

Blom, C. 1999. Adaptations to flooding stress: From plant community to molecule. Plant Biology 1:261-273.

Bodelier, P. L. E., J. A. Libochant, C. Blom, and H. J. Laanbroek. 1996. Dynamics of nitrification and denitrification in root-oxygenated sediments and adaptation of ammonia-oxidizing bacteria to low-oxygen or anoxic habitats. Applied and Environmental Microbiology 62:4100-4107.

Bodelier, P. L. E., A. G. Wijlhuizen, C. Blom, and H. J. Laanbroek. 1997. Effects of photoperiod on growth of and denitrification by *Pseudomonas chlororaphis* in the root zone of Glyceria maxima, studied in a gnotobiotic microcosm. Plant and Soil 190:91-103.

Botton, S., M. van Heusden, J. R. Parsons, H. Smidt, and N. van Straalen. 2006. Resilience of microbial systems towards disturbances. Critical Reviews in Microbiology 32:101-112.

Caffrey, J. M., N. Bano, K. Kalanetra, and J. T. Hollibaugh. 2007. Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. ISME Journal 1:660-662.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology 81:1402-1414.

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology 12:3137-3149.

Decaens, T. 2010. Macroecological patterns in soil communities. Global Ecology and Biogeography 19:287-302.

Dumbrell, A. J., M. Nelson, T. Helgason, C. Dytham, and A. H. Fitter. 2010. Relative roles of niche and neutral processes in structuring a soil microbial community. ISME Journal 4:337-345.

Erguder, T. H., N. Boon, L. Wittebolle, M. Marzorati, and W. Verstraete. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. Fems Microbiology Reviews 33:855-869.

Fierer, N. and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67:798-805.

Folke, C., C. S. Holling, and C. Perrings. 1996. Biological diversity, ecosystems, and the human scale. Ecological Applications 6:1018-1024.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal 1:19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean.

Proceedings of the National Academy of Sciences of the United States of America 102:14683-14688.

Gee, G. W. and J. W. Bauder. 1979. Particle-size analysis by hydrometer - Simplified method for routine textural analysis and a sensitivity test of measurement parameters. Soil Science Society of America Journal 43:1004-1007.

Giri, B., P. Giang, R. Kumari, R. Prasad, and A. Varma. 2005. Microbial Diversity in Soils. Microorganisms in Soils: Roles in Genesis and Functions 3:19-55.

Grinnell, J. 1917. The niche relationship of the California Thrasher. The Auk 34:427-433.

Gutknecht, J. L. M., R. M. Goodman, and T. C. Balser. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil 289:17-34.

He, S. M., F. I. Bishop, and K. D. McMahon. 2010. Bacterial community and "*Candidatus* Accumulibacter" population dynamics in laboratory-scale enhanced biological phosphorus removal reactors. Applied and Environmental Microbiology 76:5479-5487.

Henderson, S. L., C. E. Dandie, C. L. Patten, B. J. Zebarth, D. L. Burton, J. T. Trevors, and C. Goyer. 2010. Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions, and denitrification in anoxic soil microcosms amended with glucose and plant residues. Applied and Environmental Microbiology 76:2155-2164.

Hughes, J. B. 2000. The scale of resource specialization and the distribution and abundance of lycaenid butterflies. Oecologia 123:375-383.

Ikenaga, M., R. Guevara, A. L. Dean, C. Pisani, and J. N. Boyer. 2010. Changes in community structure of sediment bacteria along the Florida Coastal Everglades marsh-mangrove-seagrass salinity gradient. Microbial Ecology 59:284-295.

Jayakumar, A., G. D. O'Mullan, S. W. A. Naqvi, and B. B. Ward. 2009. Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. Microbial Ecology 58:350-362.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Kent, A. D., A. C. Yannarell, J. A. Rusak, E. W. Triplett, and K. D. McMahon. 2007. Synchrony in aquatic microbial community dynamics. ISME Journal 1:38-47.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology 55:485-529.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. Elsevier Science, Amsterdam.

Loreau, M., N. Mouquet, and A. Gonzalez. 2003. Biodiversity as spatial insurance in heterogeneous landscapes. Proceedings of the National Academy of Sciences of the United States of America 100:12765-12770.

Martens-Habbena, W., P. M. Berube, H. Urakawa, J. R. de la Torre, and D. A. Stahl. 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461:976-981.

Moin, N. S., K. A. Nelson, A. Bush, and A. E. Bernhard. 2009. Distribution and diversity of archaeal and bacterial ammonia oxidizers in salt marsh sediments. Applied and Environmental Microbiology 75:7461–7468.

Naeem, S. and S. B. Li. 1997. Biodiversity enhances ecosystem reliability. Nature 390:507-509.

Naeem, S. and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: Deriving solutions to a seemingly insurmountable problem. Ecology Letters 6:567-579.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology 10:2966-2978.

Nogales, B., K. N. Timmis, D. B. Nedwell, and A. M. Osborn. 2002. Detection and diversity of expressed denitrification genes in estuarine sediments after reverse transcription-PCR amplification from mRNA. Applied and Environmental Microbiology 68:5017-5025.

Peralta, A. L., J. W. Matthews, and A. D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology 76:4207-4215.

Petchey, O. L. and K. J. Gaston. 2006. Functional diversity: Back to basics and looking forward. Ecology Letters 9:741-758.

Pett-Ridge, J. and M. K. Firestone. 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Applied and Environmental Microbiology 71:6998-7007.

Philippot, L. 2002. Denitrifying genes in bacterial and archaeal genomes. Biochimica Et Biophysica Acta-Gene Structure and Expression 1577:355-376.

Picek, T., M. Simek, and H. Santruckova. 2000. Microbial responses to fluctuation of soil aeration status and redox conditions. Biology And Fertility Of Soils 31:315-322.

Prosser, J. I. 2007. The Ecology of Nitrifying Bacteria.*in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle. Elsevier, Oxford.

Prosser, J. I. and G. W. Nicol. 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environmental Microbiology 10:2931-2941.

Rees, G. N., D. S. Baldwin, G. O. Watson, S. Perryman, and D. L. Nielsen. 2004. Ordination and significance testing of microbial community composition derived from terminal

restriction fragment length polymorphisms: application of multivariate statistics. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 86:339-347.

Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology 69:5974-5982.

Rich, J. J. and D. D. Myrold. 2004. Community composition and activities of denitrifying bacteria from adjacent agricultural soil, riparian soil, and creek sediment in Oregon, USA. Soil Biology & Biochemistry 36:1431-1441.

Rousk, J., E. Baath, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 4:1340-1351.

Shelford, V. E. 1913. Animal Communities in a Temperate America.

Silvertown, J., M. Dodd, D. Gowing, C. Lawson, and K. McConway. 2006. Phylogeny and the hierarchical organization of plant diversity. Ecology 87:S39-S49.

Simek, M. and J. E. Cooper. 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. European Journal of Soil Science 53:345-354.

Smith, J., C. Wagner-Riddle, and K. Dunfield. 2010. Season and management related changes in the diversity of nitrifying and denitrifying bacteria over winter and spring. Applied Soil Ecology 44:138-146.

Soberon, J. 2007. Grinnellian and Eltonian niches and geographic distributions of species. Ecology Letters 10:1115-1123.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology 90:441-451.

Swan, B. K., C. J. Ehrhardt, K. M. Reifel, L. I. Moreno, and D. L. Valentine. 2010. Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. Applied and Environmental Microbiology 76:757-768.

Wallenstein, M. D., D. D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications 16:2143-2152.

Wang, D. L. and D. W. Anderson. 1998. Direct measurement of organic carbon content in soils by the Leco CR-12 Carbon Analyzer. Communications in Soil Science and Plant Analysis 29:15-21.

Worthen, W. B. 1996. Community composition and nested-subset analyses: Basic descriptors for community ecology. Oikos 76:417-426.

Yannarell, A. C. and E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology 71:227-239.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. Microbiology and Molecular Biology Reviews 61:533-616.

TABLES

Table 3.1. Average (± standard deviation) and minimum and maximum (maximum, minimum) values of environmental factors analyzed from soil samples collected along the moisture gradient. (a) Soil moisture (%), soil organic matter (% total C, % total N, % organic matter (OM)), pH and soil texture (% sand, % silt, % clay) were measured over 4 transects. Soil nutrient data was collected from a one date (June) and soil texture data was analyzed from composite soil samples collected over 4 months (June – September). (b) Soil moisture content (%) was averaged over each transect for each month.

(a)

Plot	Moisture (%)	Total C (%)	Total N (%)	OM (%)	pH	Sand (%)	Silt (%)	Clay (%)
A	11.6 ± 6.4 (7.9, 21.1)	0.97 ± 0.35 (0.64, 1.47)	0.09 ± 0.03 (0.05, 0.14)	1.78 ± 0.64 (1.17, 2.68)	5.59 ± 0.76 (4.95, 6.70)	61 ± 24 (25, 75)	21 ± 17 (11, 46)	18 ± 7 (14, 29)
B	16.1 ± 5.3 (14.5, 17.2)	0.78 ± 0.17 (0.67, 1.03)	0.07 ± 0.02 (0.06, 0.09)	1.42 ± 0.31 (1.22, 1.88)	5.90 ± 0.37 (5.53, 6.40)	52 ± 35 (1, 76)	32 ± 36 (10, 85)	16 ± 3 (14, 21)
C	25.3 ± 8.6 (22.7, 27.4)	1.64 ± 0.22 (1.46, 1.93)	0.17 ± 0.02 (0.15, 0.19)	3.00 ± 0.40 (2.67, 3.53)	5.43 ± 0.16 (5.28, 5.65)	13 ± 3 (11, 18)	44 ± 1 (42, 45)	43 ± 2 (40, 45)
D	46.0 ± 6.0 (44.5, 47.1)	2.38 ± 0.05 (2.34, 2.46)	0.23 ± 0.01 (0.22, 0.25)	4.35 ± 0.09 (4.28, 4.48)	7.63 ± 0.11 (7.48, 7.73)	3 ± 2 (1, 6)	41 ± 8 (35, 52)	57 ± 8 (46, 64)

(b)

Plot	June	July	August	September
A	15.29 ± 3.11 (11.05, 24.53)	10.90 ± 3.61 (6.71, 21.68)	7.50 ± 2.92 (4.36, 16.26)	12.71 ± 3.07 (8.89, 21.88)
B	19.59 ± 1.02 (17.23, 21.79)	16.91 ± 0.69 (14.91, 18.04)	7.80 ± 0.59 (6.27, 9.12)	20.19 ± 0.71 (18.19, 21.20)
C	28.23 ± 1.95 (22.80, 32.05)	22.51 ± 1.81 (17.36, 25.88)	14.53 ± 0.57 (13.54, 15.93)	35.83 ± 1.70 (30.95, 38.82)
D	41.04 ± 0.60 (39.28, 42.01)	43.85 ± 1.82 (40.83, 48.72)	52.70 ± 2.14 (47.46, 57.95)	51.15 ± 2.35 (47.82, 54.48)

Table 3.2. Summary of ANOSIM R values for all pairwise tests for each microbial community (denitrifiers – based on *nosZ* gene, ammonia oxidizers – based on archaeal *amoA* gene, and plant cover) comparison between location along moisture gradient (A through D: dry to saturated plots). ANOSIM R values closer to 1 indicate community dissimilarity. Pairwise tests where ANOSIM R values were greater than 0.4 were considered significantly different groups and nonrandom at $P < 0.05$.

(a) Denitrifiers Global R = 0.739

Plot	**A**	**B**	**C**	**D**
A	0			
B	0.177	0		
C	0.479	0.740	0	
D	0.719	1	1	0

(b) Ammonia oxidizers Global R = 0.760

Plot	**A**	**B**	**C**	**D**
A	0			
B	0.104	0		
C	0.740	1	0	
D	0.625	1	1	0

(c) Plants Global R = 0.833

Plot	**A**	**B**	**C**
A	0		
B	0.740	0	
C	0.667	1	0

FIGURES

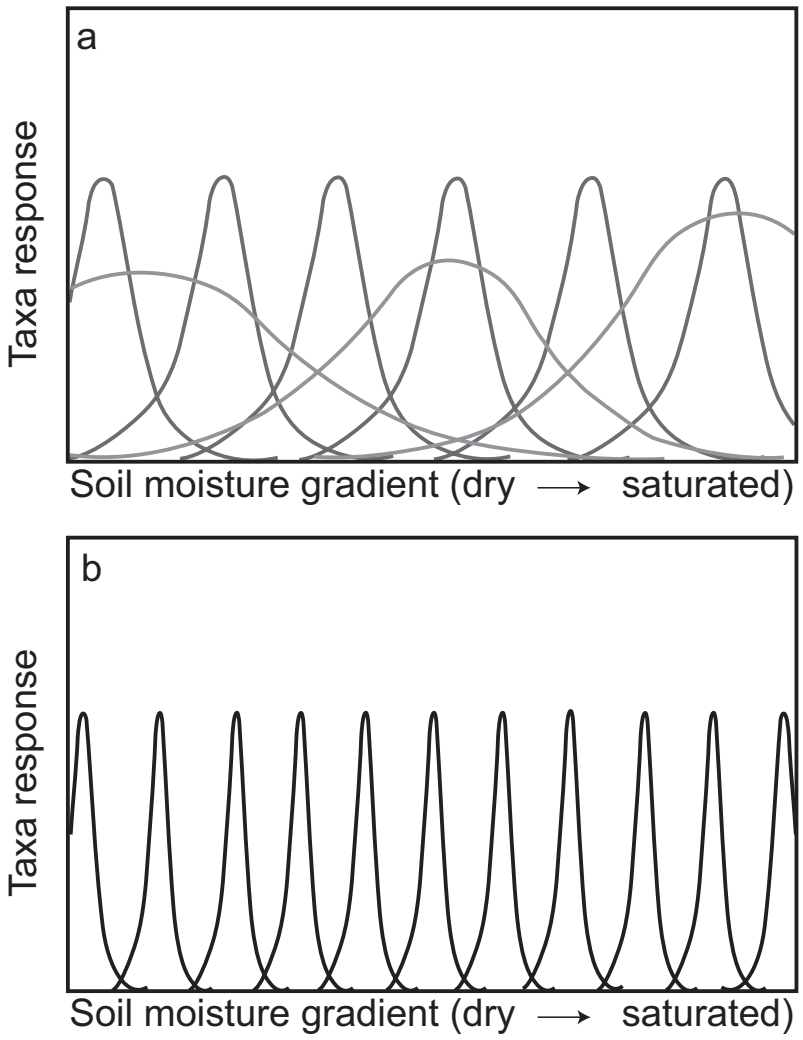


Figure 3.1. Potential microbial taxa responses along a moisture gradient. Denitrifier taxa (a) are predicted to have a broader distribution along the moisture gradient than the ammonia oxidizer taxa (b). Denitrifier taxa are expected to be narrowly and broadly distributed, displaying more habitat overlap along the gradient, whereas ammonia oxidizer taxa are more narrowly distributed, exhibiting more taxa turnover (spatial variation in composition).

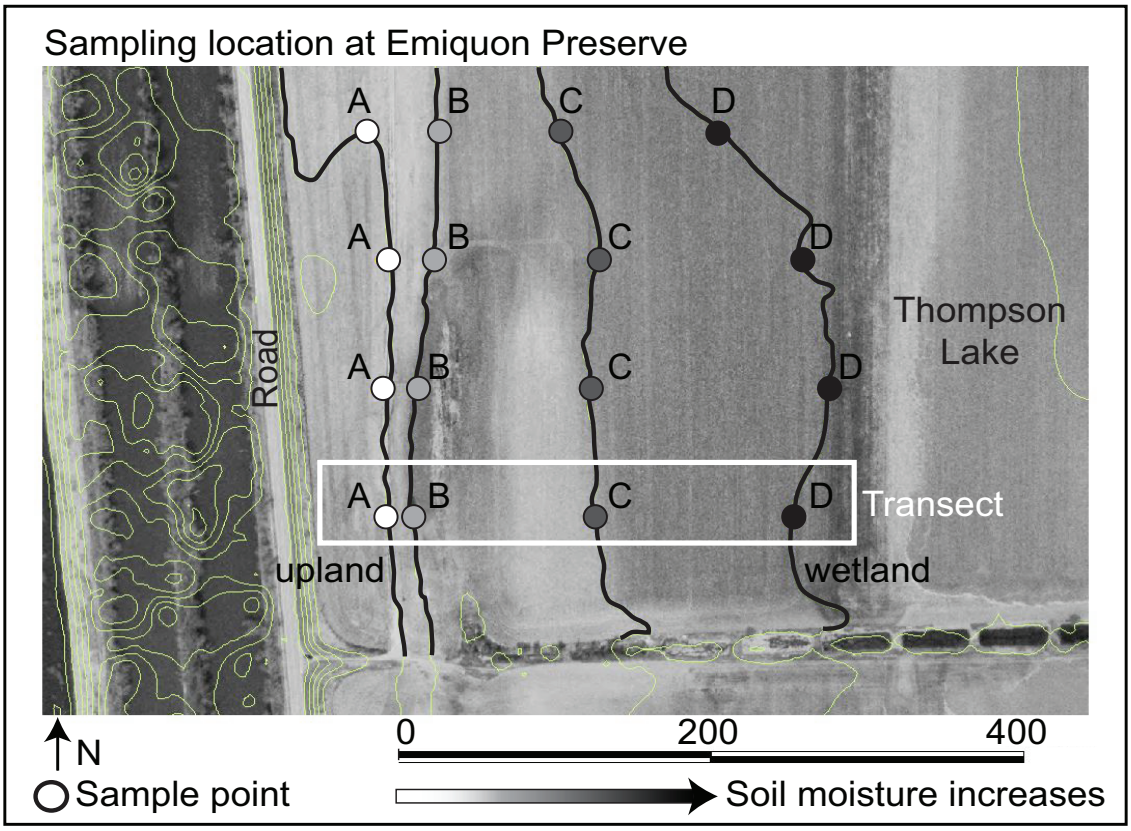


Figure 3.2. Sampling design at Emiquon Preserve. Black curves represent 1-m elevation contours. Four plots were established along each of the four transects spanning upland (white circles) to completely saturated (black circles) plots. Water depth varies at the wet-dry transition plots (plot C), while plots at the end of the gradient (plot D) remain submerged throughout the season. Soil samples and plant cover survey were collected at each location monthly from June to September 2008. The template map was obtained from The Nature Conservancy and U.S. Geological Survey.

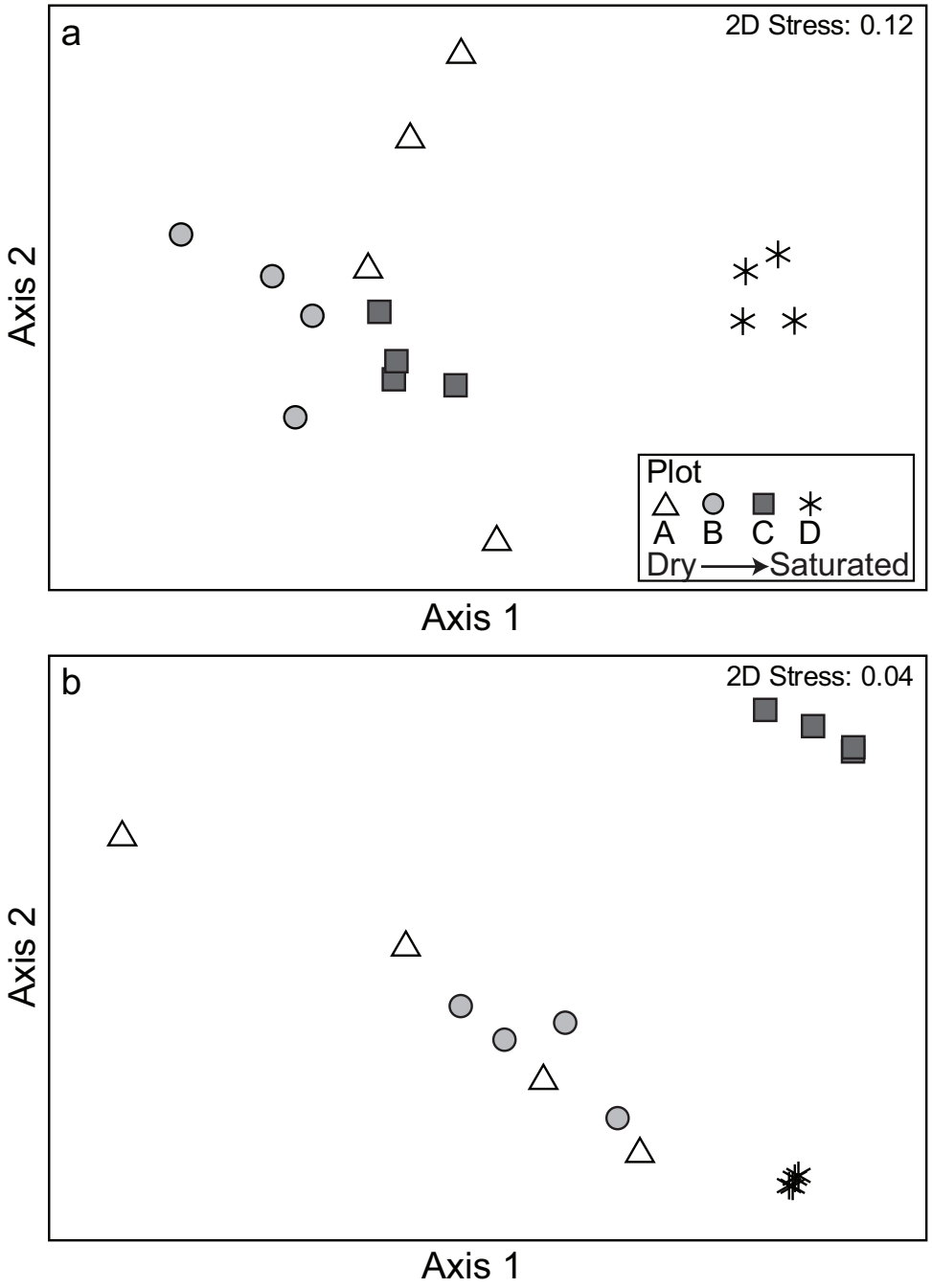


Figure 3.3. Non-metric multidimensional scaling plot of microbial community composition (averaged over four months within each plot) along the moisture gradient at Emiquon Preserve. Symbols are colored white to black and differ in shape to represent samples along an increasing moisture gradient. Each point represents community composition of the denitrifiers (a) and ammonia oxidizers (b) based on T-RFLP relative fluorescence.

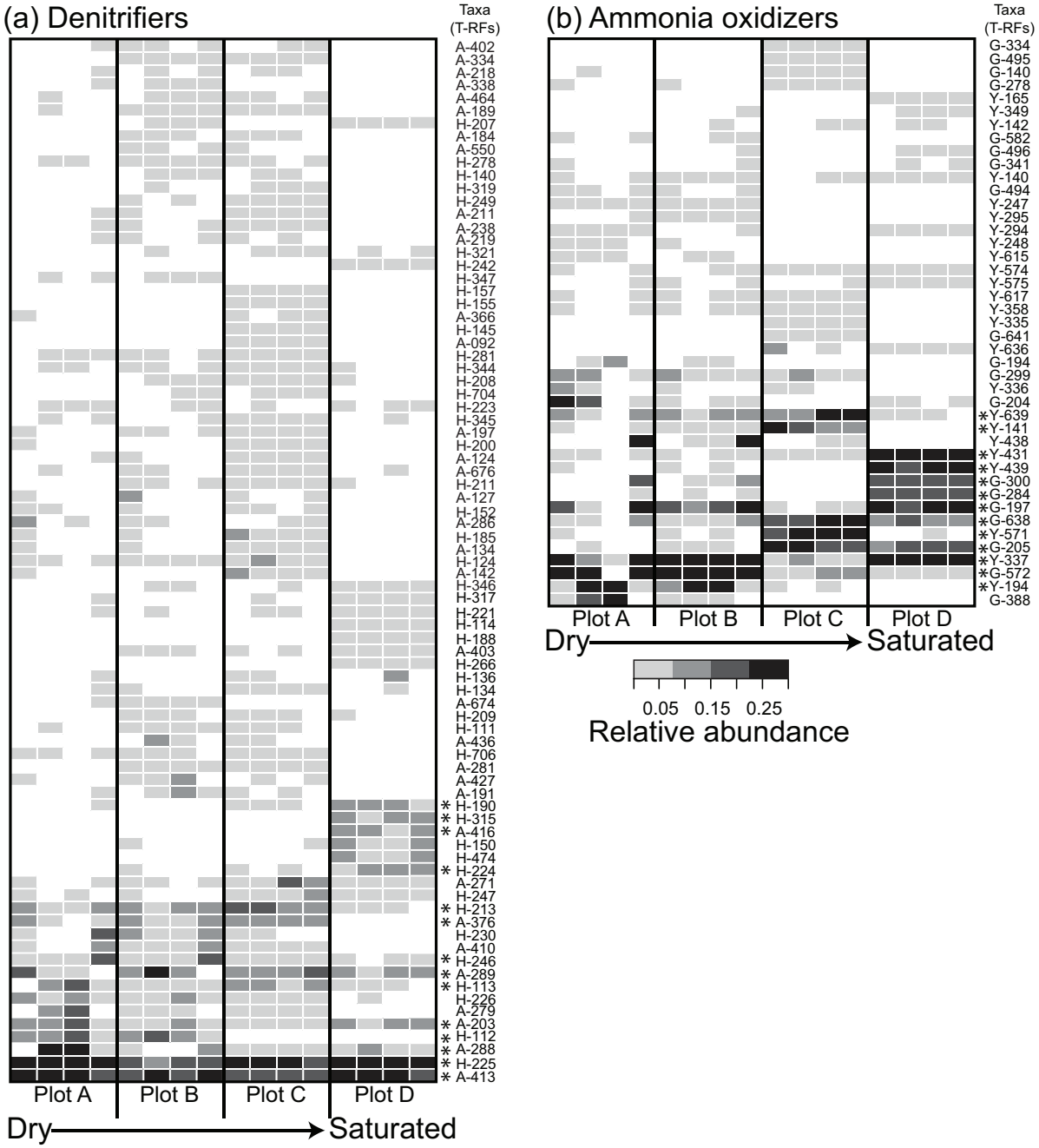
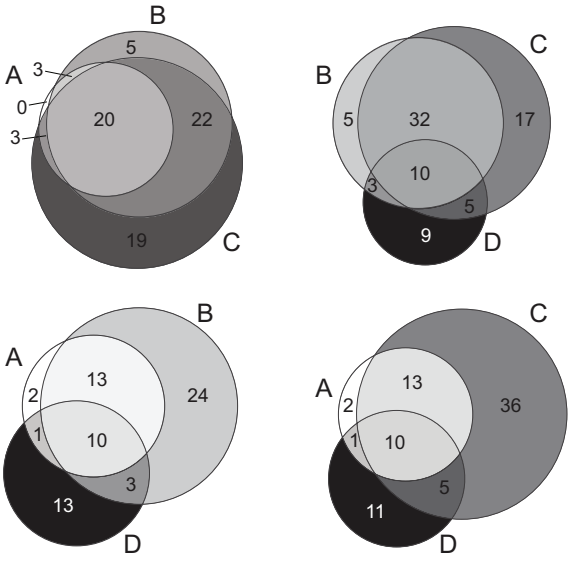


Figure 3.4. Heat map based on terminal restriction fragments (T-RFs) of denitrifier (a) and ammonia oxidizer (b) taxa. Ordering of T-RFs was based on a hierarchical cluster analysis based on the similarity of response to the environmental gradient. T-RFs observed in four or more plots along the gradient are included. Rows represent T-RFs and columns represent the four replicate sampling plots located at each discrete location along the moisture gradient from dry to saturated conditions (Plots A-D along 4 replicate transects). Scale bar represents relative abundance of each T-RF within soil communities. An asterisk (*) indicates T-RFs that strongly contribute (~15 – 30%) to within-plot similarity for a discrete location along the moisture gradient based on similarity percentages routine (SIMPER).

(a) Denitrifiers



(b) Ammonia oxidizers

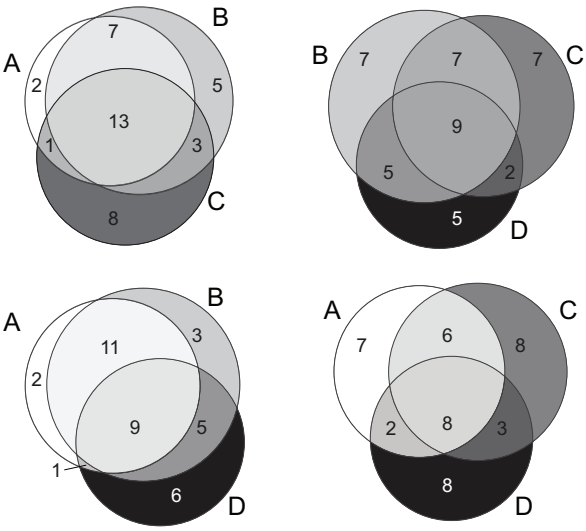


Figure 3.5. Three-way Venn diagrams based on denitrifier (a) and ammonia oxidizer (b) taxa (T-RFs presence/absence) used to visualize taxa distribution and overlap within plots (A through D) along the moisture gradient (dry to saturated). The relationships within and among plots along the gradient were scaled relative to the total number of taxa observed for each functional group (denitrifier: 81, ammonia oxidizer: 43). Number of taxa observed within each plot and within overlapping plots along the gradient is indicated on each Venn diagram. A taxon was considered present at any position (A-D) if it was detected in two out of four replicate plots along the gradient.

CHAPTER 4

ABIOTIC CORRELATES OF MICROBIAL COMMUNITY STRUCTURE AND FUNCTION VARY WITHIN WETLANDS

ABSTRACT

Understanding the relationship among community structure, ecosystem function, and local environmental conditions is fundamental to the study of ecology at all scales. To better understand microbial community structure-function relationships, we investigated relationships among nitrogen (N) cycling microbial functional groups and their activities under varied soil conditions. We were interested in long-term compared to short-term influences of environmental factors on community composition and activity related to N cycling functions. Our objective was to investigate patterns in denitrifier and ammonia oxidizer community structure and function along environmental gradients that differed substantially within each wetland site. Denitrifier and ammonia oxidizer functional groups have contrasting sensitivities to oxygen levels (and other soils factors), and variability in environmental tolerance (both within and among functional groups) can result in differential microbial responses to the same environmental gradient. Microbial structure-function relationships were compared at two restored wetlands in Illinois (site IL-1, IL-2) and two natural wetlands in Michigan (site MI-1, MI-2), where sites varied in soil fertility along a hydrologic gradient (upland to wetland). At each site, four transects were established perpendicular to the shore of the pond/lake, and four plots along each transect were placed along a hydrologic (upland to wetland) gradient. Soil chemistry and microbial community composition and abundance (structure) of denitrifiers and ammonia oxidizers (assessed by molecular analysis of functional genes) were analyzed along with potential denitrification and

nitrification activities (function). In addition to soil moisture varying along the environmental gradient, soil organic matter, soil pH, inorganic N levels and soil texture changed to various degrees at each site. Denitrifier and ammonia oxidizer community composition and activity were distinct between upland and wetland plots at all sites (PERMANOVA – plot, denitrification: IL-1: R^2 = 0. 3627, P = 0.0010; IL-2: R^2 = 0.4043, P = 0.0010; MI-1: R^2 = 0.5528, P = 0.0010; MI-2: R^2 = 0.4134, P = 0.0010); nitrification: IL-1: R^2 = 0.2975, P = 0.0010; IL-2: R^2 = 0.5314, P = 0.0010; MI-1: R^2 = 0.3402, P = 0.0010; MI-2: R^2 = 0.3461, P = 0.0010). In addition, denitrifier and ammonia oxidizer community composition were relatively constant, while microbial activity decreased over time at most sites. Partial least squares regression analyses suggest that local environmental factors (e.g., soil moisture, texture) contributed more to microbial function than microbial community composition or functional gene abundance ([*nosZ*] or [*amoA*]) (denitrification – R^2 = 72.4 %; nitrification – R^2 = 67.2 %). In addition, ammonia oxidizer community composition influenced potential nitrification rate (PSLR, R^2 = 38.4 %) more than denitrifier community composition contributed to potential denitrification rate (PLSR, R^2 = 16.4 %); suggesting possible differences between the role of microbial community composition in affecting microbial activity. Our ability to predict microbial responses to environmental change can improve by understanding the context-dependence of microbial structure-function relationships .

INTRODUCTION

Understanding the link between community structure and ecosystem function is fundamental to the study of ecology at all scales. Describing a community in terms of functional traits rather than phylogenetically-based approaches can provide information on how changes in

communities might influence ecosystem functions (Naeem and Wright 2003, Petchey and Gaston 2006). Microbial systems are ideal for addressing relationships between community structure and ecosystem functioning because taxa can be linked directly to specific functions of interest (Jessup et al. 2004). Microbial functions can be measured by comparing nutrient transformation processes carried out by resident microbes among samples (Gutknecht et al. 2006, Wallenstein et al. 2006, Zak et al. 2006). The correlation between microbial community composition and function can be generally tested using environmental treatments through manipulative experiments or natural environmental gradients (Reed and Martiny 2007, Swan et al. 2010).

Microbial taxa capable of performing the same biogeochemical transformations have been characterized, in some cases, as functionally redundant or functionally dissimilar. Communities that contain functionally redundant taxa can maintain rates of biogeochemical processes even when the composition of functional groups shift due to environmental change (Naeem et al. 2002). Distinct microbial communities subjecte to the same environmental conditions (i.e., substrate, temperature) may exhibit different rates of biogeochemical transformations as a consequence of their community composition (Balser and Wixon 2009, DeAngelis et al. 2010, Reed and Martiny 2007, Strickland et al. 2009). This 'functional dissimilarity' hypothesis assumes that microbial community composition and environmental conditions, together, control ecosystem process rates (Balser and Firestone 2005, Strickland et al. 2009). A previous study demonstrated that microbial communities that differed in initial composition varied in decomposition rates when given the same type of plant litter. Different mineralization rates were likely dependent on environmental history of these resident communities (Strickland et al. 2009). Another study demonstrated that the temperature response of bacterial communities, measured as respiration rates, varied across a latitudinal gradient

(Balser and Wixon 2009). From past studies, it is evident that changes in ecosystem-level processes may be driven by the following mechanisms: (i) the direct influence of environmental variability, where short-term environmental changes may alter activity but not composition of a community; and (ii) indirect influence of environmental variability on ecosystem functions accomplished through changes in the composition of microbial assemblages, describing a scenario where variation in environmental conditions has long-term effects, resulting in shifts in community composition and therefore changes in function (Reed and Martiny 2007). Within an environmental gradient, short-term environmental changes can be characterized by nutrient and soil moisture dynamics that frequently change on the order of days to months. In addition, long-term changes are related to soil factors that are more resistant to change in the short-term, such as soil texture, organic matter, and hydrologic regime. More work is needed to idenitify whether the restoration actvities change microbial communities and their associated ecosystem functions.

Examining microorganisms involved in nitrogen (N) cycling transformations can provide insight into structure-function relationships. The majority of N cycling transformations are microbially-mediated and well-characterized (Francis et al. 2007, Wallenstein et al. 2006). Nitrate (NO_3^-) removal functions of ecosystems occur via the denitrification pathway ($NO_3^- \rightarrow N_2$), while N is internally cycled via nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$), among other transformations. Both of these transformations are sensitive to oxygen concentration, which is strongly influenced by soil moisture (Francis et al. 2007, Kowalchuk and Stephen 2001, Wallenstein et al. 2006). Microbial functional groups associated with denitrification and nitrification can range in response to local environmental factors such as soil pH, temperature, soil inorganic N and organic matter content (Cavigelli and Robertson 2000, Nicol et al. 2008,

Wertz et al. 2007). Physiological differences among microbial functional groups contribute to the varied sensitivity of communities to abiotic factors in the environment (Jayakumar et al. 2009).

In a previous study (Chapter 3), we demonstrated that composition of denitrifier and ammonia oxidizer communities vary over a moisture gradient due, in part, to differences in taxon-level responses to the local soil environment. Denitrifier taxa were observed more frequently over a wider moisture range compared to ammonia oxidizer taxa (Chapter 3). In the present study, we examined the relationship among microbial community structure (represented as community composition) and function (measured by potential activity assays), and investigated the influence of abiotic soil factors on the microbial structure-function relationships within wetland sites. Spatially and temporally variable environmental gradients can be used to identify abiotic factors that are significantly associated with microbial community composition and activity on a general or more site-specific basis. Through sampling both restored and natural wetlands with varied fertility gradients but similar hydrologic differences, we can test the degree to which microbial functions are influenced by contemporary changes in environmental conditions (short-term response) or historical and contemporary changes in environmental conditions (long-term response where changes in community composition contribute to changes in function). Our specific objective was to compare denitrifier and ammonia oxidizer community composition and activity along hydrologic gradients within wetland ecosystems. Along with sensitivity to oxygen concentration, ammonia oxidizer community composition and activity are particularly sensitive to soil pH (Nicol et al. 2008, Prosser 2007). In addition, denitrifier community composition and activity are particularly sensitive to carbon availability as well as oxygen availability (Jayakumar et al. 2009, Vitousek and Howarth 1991, Weier et al. 1993). This study provides the unique opportunity to compare the relative importance of historical

environmental gradients to short-term changes in environmental variability between N cycling microbial functional groups. We assumed that microbial community structure was a function of the environment, while microbial activity was a function of community composition and the environment. We predicted that the hydrologic gradient within wetland sites would result in shifts in composition and activity in response to long-term environmental changes (associated with hydrologic gradient), while microbial responses to short-term environmental changes (associated with ammonium, nitrate, moisture) would result in altered activity but no shift in community structure. Predicting microbial response to future environmental change can be improved by understanding how the relationship between microbial community structure and function vary in response to natural environmental gradients.

MATERIALS AND METHODS

Site description

This study was conducted at two restored wetlands in Illinois: (IL-1) Champaign County Soil and Water District's St. Joseph Wetland (Champaign County, Illinois) and (IL-2) The Nature Conservancy's Emiquon Preserve (Fulton County, Illinois) and two wetland sites (MI-1, MI-2) adjacent to Middle Crooked Lake at Lux Arbor Reserve managed by Michigan State University's W.K. Kellogg Biological Station. The St. Joseph Wetland and Emiquon Preserve were previously under conventional agriculture prior to the beginning of restoration in 2006 and 2007, respectively. The wetlands surrounding Middle Crooked Lake have not undergone land use change and are not actively managed. The hydrology at Emiquon Preserve and Lux Arbor Reserve fluctuates in response to changes in lake levels due to precipitation. The hydrology at the St. Joseph Wetland fluctuates in response to precipitation as a consequence of changes in the

size of the temporary pond as well as flooding by an adjacent river. Site details are summarized in Table 4.1.

Sample collection

Four transects (Illinois wetlands: 20 m apart; Michigan wetlands: 10 m apart) were laid out perpendicular to the shoreline of the pond/lake at each site. Four plots (A to D) along each transect were positioned along an upland to wetland gradient, and each plot was established based on distinct changes in plant community composition (Fig. C.1). Topography and plant community composition were used as proxies for changes in moisture levels at each site. Changes in hydrology, resulting in changes in soil moisture, strongly structure wetland plant communities (Keddy 2000). At the Illinois wetlands, samples were collected within 3 1-m^2 quadrats within each plots along the hydrologic gradient. Within each plot, cover class of each plant species was assigned (<1%, 1-5%, 6-25%, 26-50%, 51-75%, 76-95%, or 96-100%) and recorded within a 1-m^2 quadrat to survey herbaceous vegetation. No vegetation was present in the saturated plots (plot D at each transect) at Emiquon Preserve (IL-2 site). At the Michigan wetlands, cover class of plant species was estimated at 1-2 1-m^2 quadrats at each plot along the hydrologic gradient and averaged to calculate species percent cover at each plot along the environmental gradient for all transects. Plant community composition significantly differed along the environmental gradient at all sites (PERMANOVA: IL-1 $R^2 = 0.6624$, $P = 0.0010$; IL-2 $R^2 = 0.6638$, $P = 0.0010$; MI-1 $R^2 = 0.7021$ $P = 0.0010$; MI-2 $R^2 = 0.5921$, $P = 0.0010$) (Table C.1, Fig. C.2). Plant species composition shifted from upland species to wetland obligate species along the upland to wetland transects.

Soil samples were collected monthly from June to August to assess short-term changes in microbial community composition, abundance and activity. Short-term changes were assumed to

be changes in soil moisture, ammonium and nitrate that varied monthly within each wetland (Table C.2). Long-term changes were assumed to be the overall influence of hydrology combined with less temporally variable soil factors (soil organic carbon and nitrogen, and soil pH) (Table C.2). Soil samples were collected at each plot within a 1-m^2 quadrat. Six soil cores (3 cm diameter) were collected to a 12 cm depth, combined, and homogenized. Plot B at MI-1 site was very rocky making it difficult to sample using the core method, therefore, samples were collected using a hand trowel and collected to 12 cm. Samples were transported on ice, and stored at 4°C prior to processing in the laboratory. A subsample was collected for storage at -80 °C for microbial analysis.

Soil chemical analyses

Gravimetric soil moisture was determined from a 20-30 g subsample from soils collected for each plot on each sampling date at all sites. Field moist soil was dried at 105 ºC for 24 hours and moisture content was calculated from the proportion of water (by weight) to oven-dried soil. From each sample, a subsample of air-dried soil was ground into a fine powder and analyzed to determine total organic matter (total organic C and total N). Elemental analyses of C and N were completed using combustion methods (ECS 4010, COSTECH Analytical Instruments, Valencia, CA, USA). Soil pH was determined for a 5 g sample analyzed using a 1:1 soil:water ratio on triplicate readings and the average pH was reported. In addition, ~5 g of field moist soil was extracted with 2 M KCl, and available ammonium (NH_4^+) and nitrate (NO_3^-) were analyzed in the extracts based on colorimetric analyses using an auto analyzer (Lachat Instruments/Hach Company, Loveland, CO). For texture analysis, soil samples collected at each date were combined for each plot along the upland to wetland gradient. Texture analysis was performed on a composite 50 g sample from each plot using the hydrometer method (Gee and Bauder 1979).

IL-1 was characterized as a high fertility site, while the IL-2, MI-1 and MI-2 sites were lower in site fertility (lower SOM, inorganic N). The soil pH gradient was relatively large at IL-2 and MI-1, but narrow at the IL-1 and MI-2 sites. In addition, the IL-1 site had more finely textured soils, while the other wetland sites changed from fine to coarsely textured from upland to wetland plots (Table C.2 & C.3).

Potential denitrification assay

On fresh soil samples, denitrification potential of the soil microbial community was estimated using the acetylene inhibition method (Royer et al. 2004, Tiedje et al. 1989). In 125-mL Wheaton bottles, 90 mL 2mM KNO_3 solution and 1.3 mL of chloramphenicol (100 mg ml^{-1}), and about 25 g of soil were combined. Bottles were sealed with septa-centered caps, shaken, purged with He for 5 min., and vented prior to beginning the assay. Prior to gas sampling, each bottle was shaken 5 minutes prior to sampling headspace to equilibrate N_2O in aqueous and sediment phases. Gas samples (15 mL) were collected at 0, 1, 2, and 3 hours.

These gas samples were analyzed for N_2O using a Shimadzu 2014 greenhouse gas analyzer. Gas standards ranging from 0.1 ppm-v to 7.46 ppm-v N_2O were generated from 99% N_2O (Grace Divisions, Deerfield, IL, USA). The N_2O concentrations of each sample per dry mass were plotted against time and the slope of this line was the potential denitrification rate (ng N_2O g^{-1} dry mass hr^{-1}). During the assay, N_2O production from each sample was linear for the majority of the samples measured. All gas samples were diluted prior to GC analysis in order for N_2O concentrations to remain within the range covered by the standard curve. Dry weight of soil samples was estimated based on gravimetric soil moisture determined for each sample.

Potential nitrification assay

Potential nitrification activity of the soil microbial community was assayed using a short-term incubation (Kandeler 1996). Approximately 5 g aliquots of soil were weighed out into two replicate 125-mL flasks. Another 5 g of soil was weighed into one control bottle. Prior to incubation, 20 mL of 1 mM $(NH_4)_2SO_4$ and 0.1 mL of 2 M $NaClO_3$ were added to prepared soil samples. The replicates were shaken for approximately 5 hours at about 130 rpm. The filtrate was collected from soils after the 5 hour incubation and from control samples stored at -20°C. This filtrate was analyzed for NO_2^--N based on a colorimetric assay. Calibration standards contained 0, 0.2, 0.4, 0.8, 1 μg NO_2^--N mL^{-1} (Kandeler 1996). Potential nitrification rate was calculated as the difference between control and incubated soil per mass of soil. Each nitrification measurement was the average of two replicates.

DNA extraction and purification

Samples were freeze-dried prior to extraction of total genomic DNA using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH). Genomic DNA was purified using a cetyl trimethyl ammonium bromide (CTAB) extraction to remove contaminating humic acids (Sambrook and Russell 2001). DNA concentration was adjusted to a standard concentration of 20 ng μl^{-1} prior to PCR and DNA-based microbial community analyses.

Denitrifier and ammonia oxidizer gene abundance

Quantitative PCR of *nosZ,* archaeal and bacterial *amoA* genes were carried out in triplicate in 10 ul reactions. The *nosZ* gene was amplified using Nos1527F, 5'- CGC TGT TC(A/C/T) TCG ACA G(C/T)C A-3' (Kloos et al. 2001) and nosZ1622R, 5'- CGC (G/A)A(C/G) GGC AA(G/C) AAG GT(G/C) CG-3' (Throback et al. 2004). The archaeal *amoA* gene was amplified using PCR primers Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3') and Arch-

amoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al. 2005); and the bacterial *amoA* gene was amplified using PCR primers amoA-1F; 5-GGGG TTTCTACTGGTGGT-3' and amoA-2R; 5-CCCCTCKGSAAAGCCTTCTTC-3' (Rotthauwe et al. 1997). PCR reactions contained 1X SYBR green master mix (Applied Biosystems Inc., Foster City, CA), 0.4 μM of each primer, 0.5 μg μl^{-1} bovine serum albumin, and 2 ul of soil DNA of known concentration. Fragments were amplified with an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of 95°C for 1 min, 56 °C for 1 min and 72 °C for 1 min. Standard curves were obtained based on serial dilutions of mixed PCR product from wetland soil samples. Reactions were analyzed on a 384-well Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems Inc., Foster City, CA).

Microbial community analyses

Composition of denitrifying microorganisms was assessed using terminal restriction fragment length polymorphism (T-RFLP) of the *nosZ* gene. In this study, "denitrifiers" refers specifically to the subset of microorganisms that carry out the last step of denitrification ($N_2O \rightarrow N_2$) catalyzed by nitrous oxide reductase, encoded by nitrous oxide reductase (*nosZ*) gene. PCR reactions to amplify the *nosZ* gene contained 50 mM Tris (pH 8.0), 250 μg of bovine serum albumin per ml, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 20 pmol of each primer, 2.5 U of *Taq* polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 μl. This gene was amplified using *nosZ*-F-1181, 5'-CGCTGTTCITCGACAGYCAG-3' and *nosZ*-R-1880, 5'-ATGTGCAKIGCRTGGCAGAA-3' to yield a 700 bp PCR product (Rich et al. 2003). The *nosZ* reverse primer was labeled with the phosphoramidite dye 6-FAM. PCR reactions were cycled with initial denaturation at 94 °C for 3 min, followed by 25 cycles of 94 °C for 45 s, 56 °C for 1 min, and 72 °C for 2 min, with a final extension carried out at 72 °C for 7 min. PCR

product from two 50 µl reactions were combined and concentrated using the Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA). Amplicons from each soil sample were digested in single-enzyme restriction digests containing *Alu*I and *Hha*I.

Ammonia monooxygenase is responsible for catalyzing the rate-limiting first step of nitrification (Kowalchuk and Stephen 2001). Community composition of ammonium oxidizing archaea was assessed using T-RFLP analysis of the *amoA* gene encoding the catalytic α-subunit of archaeal ammonia monooxygenase. Since the archaeal *amoA* gene was in higher abundance than bacterial *amoA* gene at all wetland sites, community composition was determined for this group only. PCR reactions to amplify the *amoA* gene contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of Taq polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 µl. This gene was amplified using *amoA-F*, 5'-STAATGGTCTGGCTTAGACG-3' and *amoA-R*, 5'-GCGGCCATCCATCTGTATGT-3' to yield a 650 bp amplicon (Francis et al. 2005). The *amoA* forward primer was labeled with the fluorescent dye HEX, and the *amoA* reverse primer was labeled with the fluorescent dye NED. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 53 °C for 1 min, and 72 °C for 1 min, with a final extension carried out at 72 °C for 15 min. PCR product from a single 50 µl reaction was concentrated using the Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA, USA). PCR products amplified from each soil sample were digested with *Rsa*I, generating 2 fluorescently-labeled terminal restriction fragments (T-RFs) from each amplicon.

The length and relative abundance of terminal restriction fragments (T-RFs) were analyzed by denaturing capillary electrophoresis using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 63 °C and 15 kV with a

run time of 120 minutes using POP-7 polymer. The internal size standard used for the *nosZ* and *amoA* T-RFLP analysis was the ABI GeneScan ROX 1000 size standard (Applied Biosystems, Foster City, CA). Size-calling was carried out using GeneMarker version 1.95 (SoftGenetics, State College, PA). For our analyses, each terminal restriction fragment represented a microbial taxon, and the combination of all T-RFs observed in each soil sample corresponds to the assemblage of microorganisms present. In order to account for run-to-run variations in signal detection, each T-RF was expressed as a proportion of the observed community by normalizing the signal strength (e.g. peak area) of each T-RF peak to the total fluorescence observed for each digest (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Terminal restriction fragments that exceeded a detection threshold of 100 and 300 relative fluorescence units for *nosZ* and *amoA* analysis, respectively, were included in community analyses. Normalized T-RFLP profiles produced from separate digests of each PCR product (or from different fluors, in the case of *amoA*) were concatenated prior to statistical analysis (Fierer and Schimel 2003, Peralta et al. 2010).

Statistical analyses

All wetland sites were analyzed independently since the environmental gradient was not identical at every site. Analysis of variance (ANOVA) was used to test for differences in microbial activity along the gradient over three months. Potential denitrification rates in IL-1, MI-1, and MI-2 and nitrification in MI-1 and MI-2 were log-transformed prior to running ANOVA in order to satisfy assumptions of normality. Sampling month and plot along the gradient were considered fixed factors, plot within transect was a random factor, and month was used as a repeated measure. Tukey's HSD test was carried out to determine between-group

differences in potential microbial activities. ANOVA was run in the MIXED procedure of SAS (PROC MIXED, SAS v9.2, SAS Institute).

Similarity matrices were generated separately for denitrifier and ammonia oxidizer communities by calculating the Bray-Curtis similarity coefficient for each pair of samples (Legendre and Legendre 1998). T-RF relative fluorescence data was treated as relative abundance data in our statistical procedures, as is common in microbial ecology studies (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship among microbial communities along the moisture gradient using the metaMDS function in the R Statistics Package (R Development Core Team 2011). NMDS is based on the rank order relation of dissimilarities based on the Bray-Curtis similarity coefficient, where the largest distance between points on the NMDS ordination represents the most dissimilar microbial communities. The envfit function was used to fit microbial activity and soil factors significantly associated with microbial variation (based on PERMANOVA) and overlaid on each NMDS ordination by wetland.

Two statistical methods were used to assess structure-function relationships and separate analyses were run for denitrifiers and ammonia oxidizers: partial least squares regression analysis (PLSR) and permutational multivariate analysis of variance (PERMANOVA). Partial least squares regression provides a way to assess the variation in response variables as a function of many predictors without violating major assumptions associated with using classical multiple regression analysis on ecological data (Carrascal et al. 2009). Variables are commonly non-independent and are often intercorrelated making it difficult to interpret the linear regression equation; and when the number of predictor variables exceeds or is equal to the number of samples, it is difficult to accurately calculate regression coefficients (Carrascal et al. 2009).

PLSR makes it possible to analyze the influence of a set of predictor variables that are potentially correlated and to maximize the explained variation in one or more response variables (Carrascal et al. 2009). This analysis is becoming more commonly used for multivariate regression analysis in ecological studies (Carrascal et al. 2009, Fraterrigo and Downing 2008).

The PLSR analysis was completed using the pls function in the R Statistics Package (R Development Core Team 2011). Missing data was removed prior to analysis because the pls function cannot handle missing data. Determination of the number of components to retain in a PSLR model was based on leave-one-out cross validation (LOO) using 50 leave-one-out segments (Lachenbruch and Mickey 1968). The number of components retained in the final PSLR model was based on lowest value of the root mean squared error of prediction (RMSEP) (Mevik and Wehrens 2007). The number of components retained was based on the lowest RMSEP generated out of 10 components originally considered. Three individual PLSR formulas were run to assess the influence of the local environmental and/or microbial community composition or abundance on microbial function (denitrification or nitrification): (1) function ~ community composition + environmental factors, (2) function ~ gene abundance + environmental factors, and (3) function ~ community composition. Correlation loadings (greater than 0.1) associated with each retained principal component were included in the results and discussed. The higher the loadings, the more correlated the factor is to the principal component. The second method to test for structure-function relationships assessed the relative influence of time and hydrologic gradient on microbial structure and function. Permutational (nonparametric) multivariate analysis of variance (PERMANOVA) was used to assess the effect of location along the gradient (plot) and sampling date within each wetland. The combination of PERMANOVA

results to assess community structure over time and the ANOVA to assess potential microbial activity over time was also used to examine the structure-function relationship.

PERMANOVA was also used to assess the relationship among different environmental factors and potential microbial activity on microbial community variation within each wetland (McArdle and Anderson 2001). Microbial activity was included in the PERMANOVA model to relate activity to microbial community variation. It was included in the same model as the soil factors because soil factors also influence the relationship between structure and function. To remove collinearity between variables in the PERMANOVA model, Pearson's product moment correlation was computed for all pairs of soil factors. Total organic carbon (TOC) and total nitrogen (TN) were highly correlated at all wetlands sites (IL-1: $r = 0.94$, $P<0.05$; IL-2: $r = 0.90$, $P<0.05$; MI-1: $r = 0.96$, $P<0.05$; MI-2: $r = 0.92$, $P<0.05$), so TN was removed prior to PERMANOVA. At IL-1, IL-2, and MI-1, percent sand, silt and clay were negatively correlated (sand-silt: IL-1: $r = -0.99$, $P<0.05$; sand-silt: IL-2: $r = -0.98$, $P<0.05$; MI-1: $r = -0.98$, $P<0.05$; sand-clay: IL-1: $r = -0.98$, $P<0.05$; sand-clay: IL-2: $r = -0.91$, $P<0.05$; MI-1: $r = -0.95$, $P<0.05$), so sand and silt were removed prior to PERMANOVA. At the MI-2 site, percent sand and clay were highly correlated ($r = -0.98$, $P<0.05$), so sand was removed prior to PERMANOVA. The adonis function in the R Statistics Package was used to carry out PERMANOVA (R Development Core Team 2011). The adonis function carries out sequential tests (similar to Type I sums of squares) (Oksanen et al. 2010). Only the effect of the variable entered last into the model was assessed after all other variables are accounted for in the final PERMANOVA model summary.

RESULTS

Microbial activity along the gradient

At all wetland sites, potential denitrification rate increased from upland to wetland plots while also decreasing over time. Higher fertility measured at site IL-1 supported higher potential denitrification and nitrification rates, and both functions increased from upland to wetland plots (Fig. 4.1, Table 4.2). Potential denitrification rates generally increased from upland to wetland plots at the other sites as well. Potential nitrification rates were highest in wet-dry transition plots (B) at IL-2 and decreased over time, whereas rates were very low across the gradient at Michigan sites (Fig. 4.2, Table 4.2).

Denitrifier structure-function relationship along the gradient within each wetland

There was no consistent trend in *nosZ* gene abundance along the hydrologic gradient. The *nosZ* gene abundance was significantly different along the upland to wetland gradient at the IL-2 and MI-2 sites: (IL-2 plot: $F_{(3,33)} = 6.13$, P = 0.0020; MI-2 plot: $F_{(3,32)} = 3.36$, $P = 0.0306$), and the *nosZ* gene abundance significantly different over time at IL-1 and MI-1 only (IL-1 month: $F_{(2,33)} = 18.06$, $P < 0.0001$; MI-1 month: $F_{(2,27)} = 6.88$, $P = 0.0038$) (Table 4.3, Fig. 4.3).

The total number of denitrifier terminal restriction fragments (T-RFs) detected among wetland sites ranged from 62 to 94 (T-RF count: IL-1 = 62, IL-2 = 94, MI-1 = 88, MI-2 = 69). Potential denitrification rates changed over time, while denitrifier composition stayed constant at most wetland sites. At all wetland sites, location along the gradient was linked to significant differences in denitrifier community composition along the environmental gradient (PERMANOVA – plot: IL-1: $R^2 = 0.3627$, $P = 0.0010$; IL-2: $R^2 = 0.4043$, $P = 0.0010$; MI-1: $R^2 = 0.5528$, $P = 0.0010$; MI-2: $R^2 = 0.4134$, $P = 0.0010$) (Fig. 4.4, Table 4.3). Sampling date was

significantly but weakly associated with denitrifier community variation at IL-1 only (IL-1 month – $R^2 = 0.0928$, $P = 0.0040$) (Table 4.4).

The relationship between local soil factors and denitrifier community composition varied among wetland sites (Table 4.5). At the IL-1 site, distinct denitrifier communities were observed in upland compared to wetland plots, but there was no measured abiotic factor that were significantly associated with community variation (Table 4.5a). In general, soil pH was significantly associated with denitrifier community composition at wetland sites where pH ranged the most (IL-2, MI-1), whereas the presence of water (MI sites only) and soil texture were also significantly linked to community variation but to a weaker degree (Table 4.5).

Ammonia oxidizer structure-function relationship along the gradient within each wetland

There was no consistent trend in *amoA* gene abundance along the hydrologic gradient at the wetland sites. Both archaeal and bacterial *amoA* gene abundance resulted in similar responses. The *amoA* gene abundance was significantly different along the upland to wetland gradient at the IL-2 and MI-2 sites (IL-2 AOA plot: $F_{(3,33)} = 9.03$, $P = 0.0002$, IL-2 AOB plot: $F_{(3,33)} = 7.53$, $P = 0.0006$; MI-2 plot: $F_{(3,32)} = 14.85$, $P < 0.0001$, MI-2 AOB plot: $F_{(3,32)} = 6.31$, $P = 0.0017$), but the *amoA* gene abundance significantly differed over time at IL-1 and additionally along the hydrologic gradient at MI-1 sites (IL-1 AOA month: $F_{(2,32)} = 6.11$, $P = 0.0057$, IL-1 AOB month: $F_{(2,32)} = 18.91$, $P < 0.0001$; MI-1 month*plot: $F_{(5,25)} = 5.36$, $P = 0.0018$, plot: $F_{(3,25)} = 5.27$, $P = 0.0059$, MI-1 AOB plot: $F_{(3,12)} = 7.10$, $P = 0.0053$, month: $F_{(2,12)} = 8.14$, $P = 0.0058$) (Table 4.3, Fig. 4.3).

The total number of ammonia oxidizer terminal restriction fragments (T-RFs) detected among wetland sites ranged from 32 to 49 (T-RF count: IL-1 = 49, IL-2 = 49, MI-1 = 48, MI-2 = 32). Potential nitrification rates changed over time, while composition stayed constant at all

wetland sites. At all wetland sites, location along the gradient was significantly linked to differences in ammonia oxidizer community composition (PERMANOVA – plot, IL-1: R^2 = 0.2975, P = 0.0010; IL-2: R^2 = 0.5314, P = 0.0010; MI-1: R^2 = 0.3402, P = 0.0010; MI-2: R^2 = 0.3461, P = 0.0010) (Fig. 4.5, Table 4.6). Sampling date did not affect ammonia oxidizer community variation (Table 4.6).

Potential nitrification rates changed over time, except at MI-2. In addition, ammonia oxidizer composition did not significantly change over time. At IL-1, soil pH significantly related to differences in community composition along the gradient (R^2 = 0.0722, P = 0.0110) (Table 4.7a). Soil pH, TOC, ammonium and percent clay were significantly associated with ammonia oxidizer community variation along the gradient at the IL-2 site (pH: R^2 = 0.0955, P = 0.0010, TOC: R^2 = 0.0209, P = 0.0440; ammonium: R^2 = 0.0347, P = 0.0110, clay: R^2 = 0.0365, P = 0.0100) (Table 4.7b). At the MI-1 site, pH and percent clay were significant but more weakly related to ammonia oxidizer community variation between upland and wetland communities (pH: R^2 = 0.0343, P = 0.0539; clay: R^2 = 0.0351, P = 0.0360) (Table 4.7c). At the MI-2 site, soil C:N ratio, percent silt and clay were important soil factors related to upland compared to wetland differences in community composition (C:N ratio: R^2 = 0.0529, P = 0.0050; percent clay: R^2 = 0.1602, P = 0.0010; present silt: F =3.0022, R^2 = 0.0432, P = 0.0160) (Table 4.7d). Soil pH, texture and organic matter concentration, factors that did not change over the sampling period, tended to be correlated with ammonia oxidizer community variation within wetland sites (Table 4.7).

Influence of soil characteristics and microbial community on microbial activity

Potential denitrification rate was mostly influenced by environmental factors and not denitrifier community composition or abundance (Table 4.8). The environmental factors that

represented the retained principle components were the following: sand and silt for component 1 (72.4 %), moisture for component 2 (16.7 %), moisture and total organic carbon (TOC) for component 3 (4.7 %), TOC for component 4 (3.5 %), and clay for component 5 (1.0 %) (Table 4.8).

Potential nitrification rates were mainly influenced by environmental factors and not ammonia oxidizer community composition or abundance. The majority of variance explained was described in the first 4 principal components. The environmental factors that represented the principle components were the following: sand for component 1 (67.2%), moisture and TOC for component 2 (14.5%), moisture for component 3 (13.4%), and TOC for component 4 (2.7%) (Table 4.9).

The ammonia oxidizer community explained more variance in nitrification than the denitrifier community influenced denitrification. When the environmental factors were not included in model, the following denitrifier T-RF's significantly explained variation in denitrification potential: component 1 (16.4%) – T-RF H.225, H.190, and H.224, component 2 (12.8%) – T-RF H.224, A. 289, and H. 225, component 3 (11.3%) – T-RF H. 190, H.225, and H.224, and component 4 (4.3%) – T-RF H.224, A.413, and A.289 (Table 4.8). When the influence of ammonia oxidizer community on nitrification rate was assessed, two principal components significantly contributed to variance in potential nitrification rate. The ammonia oxidizer T-RF's that represented each component were T-RF G.204, Y.439, and G.58, for component 1 (38.4%) and T-RF G.572 and G.204 for component 2 (9.1%) (Table 4.9).

DISCUSSION

Environmentally-dependent structure-function relationships

The functional response of microbial communities to their local environment is challenging to generalize and varies according to the function of interest, immediate local environmental conditions, and historical environmental conditions (Reed and Martiny 2007). We observed different combinations of soil factors influenced microbial structure and function between two nitrogen cycling functional groups: denitrifiers and ammonia oxidizers. Community structure was (mostly) invariant over time, whereas microbial activity decreased over time and gene abundance varied to different degrees along the hydrologic gradient and over the sampling period within each wetland. In previous studies, denitrifier composition distinctly varied according to different land use histories, habitats, and abiotic gradients (Cavigelli and Robertson 2000, Francis et al. 2005, Nicol et al. 2008, Rich et al. 2003, Wertz et al. 2007).

Relationship among abiotic correlates and denitrifying and ammonium oxidizing community patterns

Each wetland provided a different environmental gradient that coincided with increases in soil moisture as a consequence of a hydrologic gradient. Wetlands with larger gradients in soil characteristics (i.e., wider range in abiotic factors) provided a more heterogeneous environment, resulting in more distinct patterns in community composition. For example, soil pH was significantly related to variability in denitrifier and ammonia oxidizer community composition at the wetland sites where pH ranged the most. Soil pH has been identified as an important abiotic factor influencing distribution of total bacteria and N cycling functional groups in previous studies (Fierer and Jackson 2006, He et al. 2007, Nicol et al. 2008, Rousk et al. 2010). Denitrifier and ammonia oxidizer community composition and abundance are known to vary in sensitivity

to local soil factors such as soil pH or soil organic carbon, resulting in differences in ecosystem-level response to local soil conditions. In addition, the activity of these functional groups is quite responsive to oxygen (Francis et al. 2007, Kowalchuk and Stephen 2001, Wallenstein et al. 2006).

In addition to chemical factors, soil physical factors represented by soil texture were associated with variability in microbial communities at the IL-2, MI-1, and MI-2 sites. Soil texture is often correlated with chemical changes. Soil texture can influence how water and air travel through soil, thus, influencing nutrient movement and surface area available for microorganisms to utilize (Brady and Weil 2002). In this study, soil factors that change over long timescales are influenced by the history of the local environment (soil texture, soil organic matter, pH). These factors were more important to shaping denitrifier and ammonia oxidizer community structure than soil factors that are dependent on short-term changes in precipitation or nutrient transformations (ammonium, nitrate, moisture).

Microbial structure and function along environmental gradients

When environmental conditions influence function directly or indirectly through community composition, the structure-function relationship is likely dependent on both past and contemporary environmental conditions (Chase 2010, Reed and Martiny 2007). In the present study, it was evident that community composition and activity changed over the environmental gradient at each wetland site. In addition, short-term environmental fluctuations significantly influenced microbial activity but community composition was mostly invariable over time.

Microbial function (potential nitrogen transformation rates) responded to a combination of soil factors characterized as temporally dynamic (i.e., soil moisture) and stable (i.e., soil texture, soil organic matter). Denitrifier community structure was mostly correlated with soil

variables that changed over the long-term. In contrast to the IL-1 site, where denitrifier communities may be responding to contemporary changes in their local environment, denitrifier communities in the other wetland sites may be influenced more by historical patterns of abiotic and biotic inputs and observed patterns are shaped by historical soil conditions.

Potential denitrification and nitrification rates were highest and varied more along the gradient in wetlands experiencing dynamic hydrology. For example, the IL-1 site receives floodwaters from a nearby river during early spring and large rainfall events, whereas IL-2, MI-1, and MI-2 sites do not, thus recharge of nutrients and variation in hydrology occur to a lesser degree. Long-term environmental factors (e.g., hydrology, soil texture) are stronger drivers of microbial community composition than short-term fluctuations (e.g., nitrate, ammonium, moisture). These results were similar to past studies where microbial function was strongly influenced by microbial communities collected from soil characterized by different temperature and hydrologic regimes over time (Balser and Wixon 2009, DeAngelis et al. 2010).

Periodic and episodic inputs of nutrients may contribute to a noticeable link between microbial structure and function (Lennon and Cottingham 2008). Nitrification potential was generally low at wetlands that did not receive floodwaters, but changes in nitrification potential over time in IL-2 and MI-1 may also be due to conditions possible limiting nitrification. At these sites, low soil pH at upland sites along with an unseasonably wet summer that altered typical soil moisture and redox conditions may be altering nitrification rates. Nitrification is limited below pH 6.5 (Prosser 2007). Despite non-limiting nutrient conditions during evaluation of nitrification potential, ammonia oxidizer community composition was likely affected by long-term limiting soil conditions and therefore, potential nitrification activity was relatively low, especially in the Michigan soils analyzed.

It is also possible that changes in the active microbial community and function were correlated. It is possible that RNA- compared to DNA-based community composition may be more closely related to function (Prosser 2007). In spite of the limitations frequently associated with molecular methods and activity assays, the tools used in this study provided a complementary approach for assessing potential structure and function.

Complex relationships among community structure, function, and the environment

The abiotic template varied over space due to differences in environmental conditions along the hydrologic gradient within each wetland site. This could lead to complex relationships among microbial community composition, function and a seemingly simple environmental gradient. Generalizable relationships between diversity and function have been hypothesized. For example, the redundancy hypothesis assumes that species considered to be functionally redundant carry out the same function and gain or loss in any one of these species does not influence overall function (Naeem et al. 2002, Petchey and Gaston 2006). In contrast, the rivet hypothesis assumes that species considered singular carry out a unique ecosystem function, and gain or loss of any one of these species directly affects function (Naeem et al. 2002). However, demonstration of these generalizable structure-function relationships has been complicated by the idiosyncratic nature of community response due to the fact that species within a community may exhibit a range of responses to environmental controls (Cardinale et al. 2000, Chase 2010). In order to distinguish environmental controls over the short-term and long-term, manipulative experimental approaches are needed. Environmentally-dependent structure-function relationships complicate our ability to predict microbial functional response to environmental change. In this study, we identified specific abiotic factors that influenced denitrifier and ammonia oxidizer structure and function at wetland sites described by different environmental gradients.

Understanding how the influence of environmental conditions induces long- vs. short-term responses in microbial communities and activities can help refine predictions of microbial response to future environmental change.

ACKNOWLEDGEMENTS

This chapter was completed in collaboration with Eric Johnston, Dr. Jeffrey Matthews, and Dr. Angela Kent. We would like to thank D. Blodgett, T. Hobson, S. McClure, D. Zercher at The Nature Conservancy, B. Stikkers and R. Weitekamp at the Champaign County Soil and Water District and K. Gross and P. Barry at W.K. Kellogg Biological Station for logistical support in the field and maintenance of wetland sites. We extend our thanks to S. Hamilton, L. Kinsman, J. Martina, J. O'Brien, J. Lennon, K. Chi, C. Ugarte, N. Gottel. D. Keymer, M. Lemke for assistance in the field. S. Ludmer, C. Smith, C. Mitchell, K. Woli, M. David, J. Tsai, R. Darmody, M. Masters, and Y. Mao assisted in the laboratory, and A. Davis, D. Keymer, A. Yannarell and R. Lankau offered statistical contributions. Y. Cao, J. Dalling, M. Wander, and D. Keymer provided helpful comments to earlier versions of this manuscript.

This work was supported by the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under project number ILLU 875-374. This research was also supported, in part, by the Program in Ecology, Evolution, and Conservation Biology at the University of Illinois at Urbana-Champaign.

REFERENCES

Balser, T. C. and M. K. Firestone. 2005. Linking microbial community composition and soil

processes in a California annual grassland and mixed-conifer forest. Biogeochemistry **73**:395-415.

Balser, T. C. and D. L. Wixon. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology **15**:2935-2949.

Brady, N. C. and R. R. Weil. 2002. The Nature and Properties of Soils. 13th edition. Prentice Hall, Upper Saddle Creek, New Jersey.

Cardinale, B. J., K. Nelson, and M. A. Palmer. 2000. Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos **91**:175-183.

Carrascal, L. M., I. Galvan, and O. Gordo. 2009. Partial least squares regression as an alternative to current regression methods used in ecology. Oikos **118**:681-690.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology **81**:1402-1414.

Chase, J. M. 2010. Stochastic Community Assembly Causes Higher Biodiversity in More Productive Environments. Science **328**:1388-1391.

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology **12**:3137-3149.

Fierer, N. and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

Fierer, N. and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal **67**:798-805.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal **1**:19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America **102**:14683-14688.

Fraterrigo, J. M. and J. A. Downing. 2008. The influence of land use on lake nutrients varies with watershed transport capacity. Ecosystems **11**:1021-1034.

Gee, G. W. and J. W. Bauder. 1979. Particle-size analysis by hydrometer - Simplified method for routine textural analysis and a sensitivity test of measurement parameters. Soil Science Society of America Journal **43**:1004-1007.

Gutknecht, J. L. M., R. M. Goodman, and T. C. Balser. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil **289**:17-34.

He, J. Z., J. P. Shen, L. M. Zhang, Y. G. Zhu, Y. M. Zheng, M. G. Xu, and H. J. Di. 2007. Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices (vol 9, pg 2364, 2007). Environmental Microbiology **9**:3152-3152.

Jayakumar, A., G. D. O'Mullan, S. W. A. Naqvi, and B. B. Ward. 2009. Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. Microbial Ecology **58**:350-362.

Jessup, C. M., R. Kassen, S. E. Forde, B. Kerr, A. Buckling, P. B. Rainey, and B. J. M. Bohannan. 2004. Big questions, small worlds: microbial model systems in ecology. Trends In Ecology & Evolution **19**:189-197.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Kent, A. D., A. C. Yannarell, J. A. Rusak, E. W. Triplett, and K. D. McMahon. 2007. Synchrony in aquatic microbial community dynamics. ISME Journal **1**:38-47.

Kloos, K., A. Mergel, C. Rosch, and H. Bothe. 2001. Denitrification within the genus *Azospirillum* and other associative bacteria. Australian Journal of Plant Physiology **28**:991-998.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55**:485-529.

Lachenbruch, P. A. and M. R. Mickey. 1968. Estimation of error rates in discriminant analysis. Technometrics **10**:1-11.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. Elsevier Science, Amsterdam.

Lennon, J. T. and K. L. Cottingham. 2008. Microbial productivity in variable resource environments. Ecology **89**:1001-1014.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology **82**:290-297.

Mevik, B. H. and R. Wehrens. 2007. The pls package: Principal component and partial least squares regression in R. Journal of Statistical Software **18**:1-24.

Naeem, S., M. Loreau, and P. Inchausti. 2002. Biodiversity and ecosystem functioning: the emergence of a synthetic ecological framework. Pages pp. 3-11 *in* M. Loreau, S. Naeem,

and P. Inchausti, editors. Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxform University Press, Oxford.

Naeem, S. and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: Deriving solutions to a seemingly insurmountable problem. Ecology Letters **6**:567-579.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology **10**:2966-2978.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2010. Community Ecology Package 'vegan'.

Peralta, A. L., J. W. Matthews, and A. D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology **76**:4207-4215.

Petchey, O. L. and K. J. Gaston. 2006. Functional diversity: Back to basics and looking forward. Ecology Letters **9**:741-758.

Prosser, J. I. 2007. The Ecology of Nitrifying Bacteria.*in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle. Elsevier, Oxford.

Reed, H. E. and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology **62**:161-170.

Rees, G. N., D. S. Baldwin, G. O. Watson, S. Perryman, and D. L. Nielsen. 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **86**:339-347.

Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology **69**:5974-5982.

Rotthauwe, J. H., K. P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology **63**:4704-4712.

Rousk, J., P. C. Brookes, and E. Baath. 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology & Biochemistry **42**:926-934.

Royer, T. V., J. L. Tank, and M. B. David. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. Journal of Environmental Quality **33**:1296-1304.

Sambrook, J. and D. Russell. 2001. Molecular cloning: A laboratory manual.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Swan, B. K., C. J. Ehrhardt, K. M. Reifel, L. I. Moreno, and D. L. Valentine. 2010. Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. Applied and Environmental Microbiology **76**:757-768.

Throback, I. N., K. Enwall, A. Jarvis, and S. Hallin. 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiology Ecology **49**:401-417.

Tiedje, J. M., S. Simkins, and P. M. Groffman. 1989. Perspectives on Measurement of Denitrification in the Field Including Recommended Protocols for Acetylene Based Methods. Plant and Soil **115**:261-284.

Vitousek, P. M. and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea - How can it occur? Biogeochemistry **13**:87-115.

Wallenstein, M. D., D. D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications **16**:2143-2152.

Weier, K. L., J. W. Doran, J. F. Power, and D. T. Walters. 1993. Denitrification and the dinitrogen nitrous oxide ratio as affected by soil-water, available carbon, and nitrate Soil Science Society of America Journal **57**:66-72.

Wertz, S., V. Degrange, J. I. Prosser, F. Poly, C. Commeaux, N. Guillaumaud, and X. Le Roux. 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environmental Microbiology **9**:2211-2219.

Yannarell, A. C. and E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology **71**:227-239.

Zak, D. R., C. B. Blackwood, and M. P. Waldrop. 2006. A molecular dawn for biogeochemistry. Trends In Ecology & Evolution **21**:288-295.

TABLES

Table 4.1. Description of wetland sites sampled in this study.

site ID	site	latitude	longitude	adjacent water body
IL-1	St. Joseph Wetland	40.12	-88.05	temporary pond
IL-2	Emiquon Preserve	40.34	-90.09	Thompson Lake
MI-1	Lux Arbor_1	42.48	-85.46	Middle Crooked Lake
MI-2	Lux Arbor_2	42.48	-85.46	Middle Crooked Lake

Table 4.2. Summary of analysis of variance (ANOVA) results. ANOVA of the main effects (plot along gradient and month) and the interactions of main effects was carried out on potential denitrification and nitrification rates. Effects were considered significantly different at $P < 0.05$. *Post-hoc* contrasts are found in Table S1 C.1.

(a) IL-1

log denitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	10	<0.0001
month	2, 33	259.89	<0.0001
month*plot	6, 33	8.37	<0.0001

nitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	14.52	<0.0001
month	2, 33	5.93	0.0063
month*plot	6, 33	1.5	0.2084

(b) IL-2

denitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	6.28	0.0017
month	2, 33	18.02	<0.0001
month*plot	6, 33	2.55	0.0384

nitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	24.04	<0.0001
month	2, 33	4.66	0.0165
month*plot	6, 33	2.03	0.0892

(c) MI-1

log denitrification			
effect	df	*F*-value	*P*-value
plot	3, 30	8.77	0.0003
month	2, 30	29.87	<0.0001
month*plot	5, 30	0.12	0.9869

log nitrification			
effect	df	*F*-value	*P*-value
plot	3, 30	96.14	<0.0001
month	2, 30	0.89	0.4194
month*plot	5, 30	1.16	0.3514

(d) MI-2

log denitrification			
effect	df	*F*-value	*P*-value
plot	3, 32	4.44	0.0102
month	2, 32	192.29	<0.0001
month*plot	6, 32	0.7	0.6492

log nitrification			
effect	df	*F*-value	*P*-value
plot	3, 32	1.34	0.2792
month	2, 32	0.32	0.7252
month*plot	6, 32	1.09	0.3875

Table 4.3. Summary of analysis of variance (ANOVA) results. ANOVA of the main effects (plot along gradient and month) and the interactions of main effects was carried out on *nosZ* (NOS), archaeal a*moA* (AOA), and bacterial *amoA* (AOB) gene abundance by wetland site.

(a) IL-1

NOS qPCR			
effect	df	*F*-value	*P*-value
plot	3, 33	2.32	0.0937
month	2, 33	18.06	<0.0001
month*plot	6, 33	1.27	0.2989
AOA qPCR			
effect	df	*F*-value	*P*-value
plot	3, 32	1.99	0.1348
month	2, 32	6.11	0.0057
month*plot	6, 32	1.11	0.3763
AOB qPCR			
effect	df	*F*-value	*P*-value
plot	3, 32	2.22	0.1047
month	2, 32	18.91	<0.0001
month*plot	6, 32	1.77	0.1375

(b) IL-2

NOS qPCR			
effect	df	*F*-value	*P*-value
plot	3, 33	6.13	0.0020
month	2, 33	0.74	0.4846
month*plot	6, 33	0.36	0.9003
AOA qPCR			
effect	df	*F*-value	*P*-value
plot	3, 33	9.03	0.0002
month	2, 33	1.01	0.3764
month*plot	6, 33	0.37	0.8925
AOB qPCR			
effect	df	*F*-value	*P*-value
plot	3, 33	7.53	0.0006
month	2, 33	0.9	0.4172
month*plot	6, 33	1.07	0.4020

(c) MI-1

NOS qPCR			
effect	df	*F*-value	*P*-value
plot	3, 27	1.88	0.1560
month	2 ,27	6.88	0.0038
month*plot	5, 27	1.58	0.1986
AOA qPCR			
effect	df	*F*-value	*P*-value
plot	3, 25	5.27	0.0059
month	2, 25	0.89	0.4229
month*plot	5, 25	5.36	0.0018
AOB qPCR			
effect	df	*F*-value	*P*-value
plot	3, 12	7.1	0.0053
month	2, 12	8.14	0.0058
month*plot	2, 12	2.47	0.1266

(d) MI-2

NOS qPCR			
effect	df	*F*-value	*P*-value
plot	3, 32	3.36	0.0306
month	2, 32	1.04	0.3651
month*plot	6, 32	0.32	0.9208
AOA qPCR			
effect	df	*F*-value	*P*-value
plot	3, 32	14.85	<0.0001
month	2, 32	1.15	0.3285
month*plot	6, 32	0.46	0.8324
AOB qPCR			
effect	df	*F*-value	*P*-value
plot	3, 32	6.31	0.0017
month	2, 32	1.37	0.2684
month*plot	6, 32	0.59	0.7341

Table 4.4. Summary of permutational MANOVA (PERMANOVA) results. Contribution of plot along upland to wetland gradient on denitrifier community variation at wetland sites IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d) is presented. Effects were considered significant to contributing to community variation at $P < 0.05$. (Abbrevations: Df = degrees of freedom, Sums Sqs = sums of squares, Mean Sqs = mean squares, F.Model = *F* statistic)

(a) IL-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	1.9978	0.6659	9.0693	0.3627	**0.0010**
Month	2	0.5111	0.2555	3.4800	0.0928	**0.0040**
Plot*Month	6	0.3554	0.0592	0.8068	0.0645	0.6913
Residuals	36	2.6434	0.0734	0.4799		
Total	47	5.5077	1.0000			

(b) IL-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	3.0017	1.0006	9.7310	0.4043	**0.0010**
Month	2	0.2389	0.1195	1.1619	0.0322	0.2807
Plot*Month	6	0.4828	0.0805	0.7826	0.0650	0.7722
Residuals	36	3.7016	0.1028	0.4985		
Total	47	7.4250	1.0000			

(c) MI-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	3.6999	1.2333	17.0831	0.5528	**0.0010**
Month	2	0.2079	0.1040	1.4399	0.0311	0.1658
Plot*Month	5	0.4029	0.0806	1.1161	0.0602	0.3327
Residuals	33	2.3824	0.0722	0.3560		
Total	43	6.6932	1.0000			

(d) MI-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	2.3484	0.7828	9.2301	0.4134	**0.0010**
Month	2	0.1268	0.0634	0.7477	0.0223	0.6843
Plot*Month	6	0.2370	0.0395	0.4657	0.0417	0.9970
Residuals	35	2.9683	0.0848	0.5225		
Total	46	5.6805	1.0000			

Table 4.5. Summary of permutational MANOVA (PERMANOVA) results. Contribution of soil factors and denitrification activity on denitrifier community variation at wetland sites IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d) is presented. Effects were considered significant to contributing to community variation at $P < 0.05$. (Abbreviations: CN = soil C:N ratio, TOC = total organic carbon, water = presence/absence standing water)

(a) IL-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
denitrification	1	0.1376	0.1377	1.6811	0.0250	0.1409
CN	1	0.0219	0.0219	0.2677	0.0040	0.9441
TOC	1	0.0787	0.0787	0.9616	0.0143	0.3876
nitrate	1	0.1387	0.1387	1.6936	0.0252	0.1359
ammonium	1	0.1173	0.1173	1.4320	0.0213	0.2228
water	1	0.0607	0.0607	0.7417	0.0110	0.5574
moisture	1	0.0486	0.0486	0.5936	0.0088	0.7033
pH	1	0.1935	0.1935	2.3633	0.0351	0.0559
clay	1	0.0459	0.0459	0.5607	0.0083	0.7143
Residuals	38	3.1115	0.0819	0.5649		
Total	47	5.5077	1.0000			

(b) IL-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
denitrification	1	0.0682	0.0682	0.7178	0.0092	0.5764
CN	1	0.1681	0.1682	1.7708	0.0227	0.1119
TOC	1	0.2002	0.2002	2.1085	0.0270	0.0679
nitrate	1	0.0622	0.0622	0.6553	0.0084	0.6623
ammonium	1	0.1302	0.1302	1.3707	0.0175	0.2088
water	1	0.0675	0.0675	0.7111	0.0091	0.5405
moisture	1	0.0412	0.0413	0.4344	0.0056	0.8641
pH	1	1.6032	1.6032	16.8840	0.2159	**0.0010**
clay	1	0.3257	0.3257	3.4303	0.0439	**0.0160**
Residuals	38	3.6083	0.0950	0.4860		
Total	47	7.4250	1.0000			

Table 4.5 (cont.)

(c) MI-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
denitrification	1	0.0396	0.0396	0.5133	0.0059	0.8122
CN	1	0.1037	0.1037	1.3456	0.0155	0.2298
TOC	1	0.1138	0.1138	1.4765	0.0170	0.1888
nitrate	1	0.0686	0.0686	0.8899	0.0103	0.4765
ammonium	1	0.0603	0.0603	0.7829	0.0090	0.5604
water	1	0.3296	0.3296	4.2762	0.0492	**0.0030**
moisture	1	0.0885	0.0885	1.1478	0.0132	0.3097
pH	1	0.2417	0.2417	3.1362	0.0361	**0.0140**
clay	1	0.1591	0.1591	2.0638	0.0238	0.0679
Residuals	34	2.6204	0.0771	0.3915		
Total	43	6.6932	1.0000			

(d) MI-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
denitrification	1	0.0167	0.0167	0.1984	0.0030	0.9920
CN	1	0.0830	0.0830	0.9842	0.0146	0.4136
TOC	1	0.2678	0.2678	3.1753	0.0471	**0.0180**
nitrate	1	0.0875	0.0875	1.0378	0.0154	0.3357
ammonium	1	0.1035	0.1035	1.2268	0.0182	0.2647
water	1	0.3685	0.3685	4.3695	0.0649	**0.0030**
moisture	1	0.0850	0.0850	1.0075	0.0150	0.3976
pH	1	0.0362	0.0362	0.4290	0.0064	0.8651
clay	1	0.3149	0.3149	3.7341	0.0554	**0.0090**
silt	1	0.5633	0.5633	6.6802	0.0992	**0.0010**
Residuals	36	3.0358	0.0843	0.5344		
Total	46	5.6805	1.0000			

Table 4.6. Summary of permutational MANOVA (PERMANOVA) results. Contribution of plot along upland to wetland gradient on ammnoia oxidizer community variation at wetland sites IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d) is presented. Effects were considered significant to contributing to community variation at $P < 0.05$. (Abbrevations: Df = degrees of freedom, Sums Sqs = sums of squares, Mean Sqs = mean squares, F.Model = *F* statistic)

(a) IL-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	0.5930	0.1977	6.0389	0.2975	**0.0010**
Month	2	0.1124	0.0562	1.7169	0.0564	0.0909
Plot*Month	6	0.1423	0.0237	0.7243	0.0714	0.8342
Residuals	35	1.1457	0.0327	0.5748		
Total	46	1.9934	1.0000			

(b) IL-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	4.5724	1.5241	14.7520	0.5314	**0.0010**
Month	2	0.0582	0.0291	0.2818	0.0068	0.9291
Plot*Month	6	0.2548	0.0425	0.4111	0.0296	0.9700
Residuals	36	3.7194	0.1033	0.4322		
Total	47	8.6049	1.0000			

(c) MI-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	5.0381	1.6794	6.5861	0.3402	**0.0010**
Month	2	0.4097	0.2048	0.8033	0.0277	0.6454
Plot*Month	5	0.9460	0.1892	0.7420	0.0639	0.8711
Residuals	33	8.4146	0.2550	0.5682		
Total	43	14.8084	1.0000			

(d) MI-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	3.4006	1.1336	7.5520	0.3461	**0.0010**
Month	2	0.4606	0.2303	1.5343	0.0469	0.0979
Plot*Month	6	0.7102	0.1184	0.7886	0.0723	0.8182
Residuals	35	5.2534	0.1501	0.5347		
Total	46	9.8249	1.0000			

Table 4.7. Summary of permutational MANOVA (PERMANOVA) results. Contribution of soil factors and nitrification activity on ammonia oxidizer community variation at wetland sites IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d) is presented. Effects were considered significant to contributing to community variation at $P < 0.05$. (Abbreviations: CN = soil C:N ratio, TOC = total organic carbon, water = presence/absence standing water)

(a) IL-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
nitrification	1	0.0233	0.0233	0.8354	0.0117	0.5075
CN	1	0.0375	0.0375	1.3414	0.0188	0.2378
TOC	1	0.0311	0.0311	1.1130	0.0156	0.3187
nitrate	1	0.0521	0.0521	1.8635	0.0261	0.1129
ammonium	1	0.0055	0.0055	0.1967	0.0028	0.9321
water	1	0.0050	0.0050	0.1778	0.0025	0.9191
moisture	1	0.0536	0.0536	1.9176	0.0269	0.1059
pH	1	0.1440	0.1440	5.1539	0.0722	**0.0110**
clay	1	0.0182	0.0182	0.6528	0.0092	0.5894
Residuals	37	1.0337	0.0279	0.5186		
Total	46	1.9934	1.0000			

(b) IL-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
nitrification	1	0.1749	0.1749	2.7880	0.0203	0.0669
CN	1	0.0322	0.0322	0.5120	0.0037	0.6504
TOC	1	0.1795	0.1795	2.8600	0.0209	**0.0440**
nitrate	1	0.0432	0.0432	0.6880	0.0050	0.5295
ammonium	1	0.2990	0.2990	4.7640	0.0347	**0.0110**
water	1	0.0424	0.0424	0.6750	0.0049	0.5574
moisture	1	0.1112	0.1112	1.7720	0.0129	0.1608
pH	1	0.8221	0.8221	13.1000	0.0955	**0.0010**
clay	1	0.3141	0.3141	5.0050	0.0365	**0.0100**
Residuals	38	2.3847	0.0628	0.2771		
Total	47	8.6049	1.0000			

Table 4.7 (cont.)

(c) MI-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
nitrification	1	0.4033	0.4033	1.6713	0.0272	0.0929
CN	1	0.2289	0.2289	0.9488	0.0155	0.4456
TOC	1	0.3786	0.3786	1.5691	0.0256	0.1319
nitrate	1	0.1572	0.1572	0.6516	0.0106	0.7233
ammonium	1	0.1422	0.1422	0.5894	0.0096	0.7652
water	1	0.2117	0.2117	0.8774	0.0143	0.4805
moisture	1	0.1668	0.1668	0.6913	0.0113	0.6843
pH	1	0.5083	0.5083	2.1066	0.0343	**0.0539**
clay	1	0.5194	0.5194	2.1527	0.0351	**0.0360**
Residuals	34	8.2037	0.2413	0.5540		
Total	43	14.8084	1.0000			

(d) MI-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
nitrification	1	0.2389	0.2389	1.6896	0.0243	0.1089
CN	1	0.5198	0.5198	3.6764	0.0529	**0.0050**
TOC	1	0.1827	0.1827	1.2918	0.0186	0.2308
nitrate	1	0.0828	0.0828	0.5855	0.0084	0.7562
ammonium	1	0.1022	0.1022	0.7228	0.0104	0.6014
water	1	0.1661	0.1661	1.1748	0.0169	0.2867
moisture	1	0.1506	0.1506	1.0648	0.0153	0.3876
pH	1	0.1114	0.1114	0.7878	0.0113	0.5694
clay	1	1.5738	1.5738	11.1304	0.1602	**0.0010**
silt	1	0.4245	0.4245	3.0022	0.0432	**0.0160**
Residuals	36	5.0903	0.1414	0.5181		
Total	46	9.8249	1.0000			

Table 4.8. Summary of partial least squares regression correlation loadings for each principal component retained. Soil factors or microbial taxa (T-RF) or functional gene abundance (NOSqPCR) with the highest loadings represent each principle component. The R^2 values refer to the variation explained in the function (denitrification) by the principle components. (Abbreviations: water = presence/absence standing water, TOC = total organic carbon, TN = total nitrogen, CN = soil C:N ratio)

Model: denitrification ~ environment + denitrifier community composition

Component (R^2)	Comp 1 (72.4 %)	Comp 2 (16.7 %)	Comp 3 (4.7 %)	Comp 4 (3.5 %)	Comp 5 (1.0 %)
sand	-0.841	0.421	-0.130	-0.002	0.092
silt	0.509	-0.288	-0.238	-0.218	0.567
clay	0.332	-0.133	0.368	0.220	-0.659
pH	0.016	0.021	0.018	0.054	-0.194
moisture	0.202	0.911	-0.772	0.226	-0.092
temp	0.064	-0.012	0.026	0.449	0.238
water	-0.001	0.007	-0.037	0.026	-0.012
ammonium	-0.011	0.172	-0.360	0.120	0.246
nitrate	0.018	0.037	0.007	-0.054	-0.193
TOC	0.044	0.265	1.184	-0.922	0.375
TN	0.006	0.012	0.050	-0.054	0.037
CN	-0.036	0.067	0.348	-0.121	-0.117

Model: denitrification ~ environment + *nosZ* gene abundance

Component (R^2)	Comp 1 (70.7 %)	Comp 2 (18.7 %)	Comp 3 (3.6 %)	Comp 4 (4.6 %)	Comp 5 (1.2 %)
NOSqPCR	0.006	-0.005	-0.006	-0.009	-0.004
sand	-0.832	0.464	-0.116	-0.018	0.111
silt	0.511	-0.285	-0.395	-0.029	0.573
clay	0.320	-0.179	0.512	0.047	-0.684
pH	0.018	0.012	0.040	0.025	-0.202
moisture	0.250	0.933	-0.428	-0.005	-0.022
temp	0.055	0.008	0.210	0.338	0.213
water	-0.001	0.012	-0.044	0.018	-0.013
ammonium	0.013	0.144	-0.302	0.100	0.120
nitrate	0.021	0.034	0.000	-0.068	-0.185
TOC	0.040	0.001	1.399	-0.984	0.418
TN	0.006	-0.001	0.057	-0.051	0.032
CN	-0.037	0.006	0.416	-0.177	-0.017

Table 4.8 (cont.)

Model: denitrification ~ denitrifier community composition

T-RF	Comp 1 (16.4 %)	T-RF	Comp 2 (12.8 %)	T-RF	Comp 3 (11.3 %)	T-RF	Comp 4 (4.3 %)
H.225	-0.662	H.224	-0.557	H.190	-0.470	H.224	-0.453
A.413	-0.271	A.289	-0.402	H.225	-0.440	A.413	-0.310
H.652	-0.243	A.203	-0.172	H.246	-0.302	H.105	-0.199
A.414	-0.202	H.111	-0.157	A.203	-0.291	H.221	-0.187
A.282	-0.116	H.247	-0.133	A.403	-0.263	A.414	-0.178
A.435	0.108	H.213	-0.132	A.270	-0.245	A.407	-0.155
H.221	0.150	H.227	-0.118	A.677	-0.229	H.207	0.099
A.403	0.244	H.112	-0.100	H.346	-0.216	H.223	0.108
A.550	0.266	H.652	0.096	A.463	-0.164	A.550	0.113
A.203	0.452	A.414	0.125	A.465	-0.164	H.344	0.116
H.190	0.603	A.550	0.252	A.435	-0.152	H.206	0.120
H.224	0.832	H.225	0.766	H.648	-0.140	A.270	0.131
				H.266	-0.120	H.263	0.144
				A.200	-0.113	H.346	0.154
				A.610	-0.105	H.227	0.156
				H.652	0.112	A.677	0.164
				A.289	0.131	H.225	0.167
				H.112	0.137	H.190	0.168
				H.213	0.153	H.247	0.194
				A.413	0.199	H.213	0.231
				H.111	0.216	A.403	0.310
				H.227	0.243	A.203	0.364
				H.224	0.461	H.246	0.368
						A.289	0.497

Table 4.9. Summary of partial least squares regression correlation loadings for each principal component retained. Soil factors or microbial taxa (T-RF) or functional gene abundance (AOBqPCR, AOAqPCR) with the highest loadings represent each principle component. The R^2 values refer to the variation explained in the function (nitrification) by the principle components. (Abbreviations: water = presence/absence standing water, TOC = total organic carbon, TN = total nitrogen, CN = soil C:N ratio)

Model: Nitrification ~ environment + ammonia oxidizer community composition

Component (R^2)	Comp 1 (67.2 %)	Comp 2 (14.5 %)	Comp 3 (13.4 %)	Comp 4 (2.7 %)
sand	-0.827	0.301	-0.114	-0.020
silt	0.494	-0.264	-0.083	-0.395
clay	0.333	-0.037	0.197	0.415
pH	0.018	0.019	0.009	0.118
moisture	0.131	0.998	-0.974	0.374
temp	0.055	-0.054	-0.073	0.183
water	0.001	0.004	-0.021	0.012
ammonium	-0.016	0.017	-0.345	0.015
nitrate	0.011	0.049	-0.010	0.070
TOC	0.034	0.623	0.340	-0.709
TN	0.006	0.028	0.013	-0.044
CN	-0.039	0.162	0.126	-0.060

Model: Nitrification ~ environment + bacterial and archaeal *amoA* gene abundance

Component (R^2)	Comp 1 (68.9 %)	Comp 2 (13.4 %)	Comp 3 (13.1 %)	Comp 4 (2.1 %)
AOBqPCR	0.015	-0.014	-0.004	0.050
AOAqPCR	0.019	-0.009	0.002	0.085
sand	-0.820	0.377	-0.146	-0.042
silt	0.483	-0.333	-0.021	-0.478
clay	0.337	-0.043	0.167	0.520
pH	0.016	0.031	0.017	0.136
moisture	0.198	0.873	-0.966	0.306
temp	0.051	-0.052	-0.063	0.178
water	0.001	-0.002	-0.024	0.023
ammonium	-0.014	-0.055	-0.320	0.160
nitrate	0.019	0.057	-0.005	0.068
TOC	0.085	0.625	0.299	-0.601
TN	0.007	0.028	0.014	-0.046
CN	-0.020	0.183	0.097	0.001

Table 4.9 (cont.)

Model: Nitrification ~ archaeal ammonia oxidizer community composition

T-RF	Comp 1 (38.4 %)	T-RF	Comp 2 (9.1 %)
G.204	-0.448	G.572	-0.533
Y.141	-0.268	Y.52	-0.366
G.572	-0.195	G.638	-0.267
Y.53	-0.168	Y.336	-0.260
Y.294.5	-0.113	Y.61	-0.204
G.284	0.119	Y.639	-0.202
G.197	0.165	G.299	-0.176
Y.336.5	0.195	Y.51	-0.171
G.300	0.298	G.197	-0.164
Y.61	0.313	G.58	-0.101
Y.439	0.396	Y.294.5	0.133
G.58	0.470	G.640	0.155
		Y.53	0.167
		G.300	0.180
		Y.294	0.264
		Y.141	0.308
		G.204	0.470

FIGURES

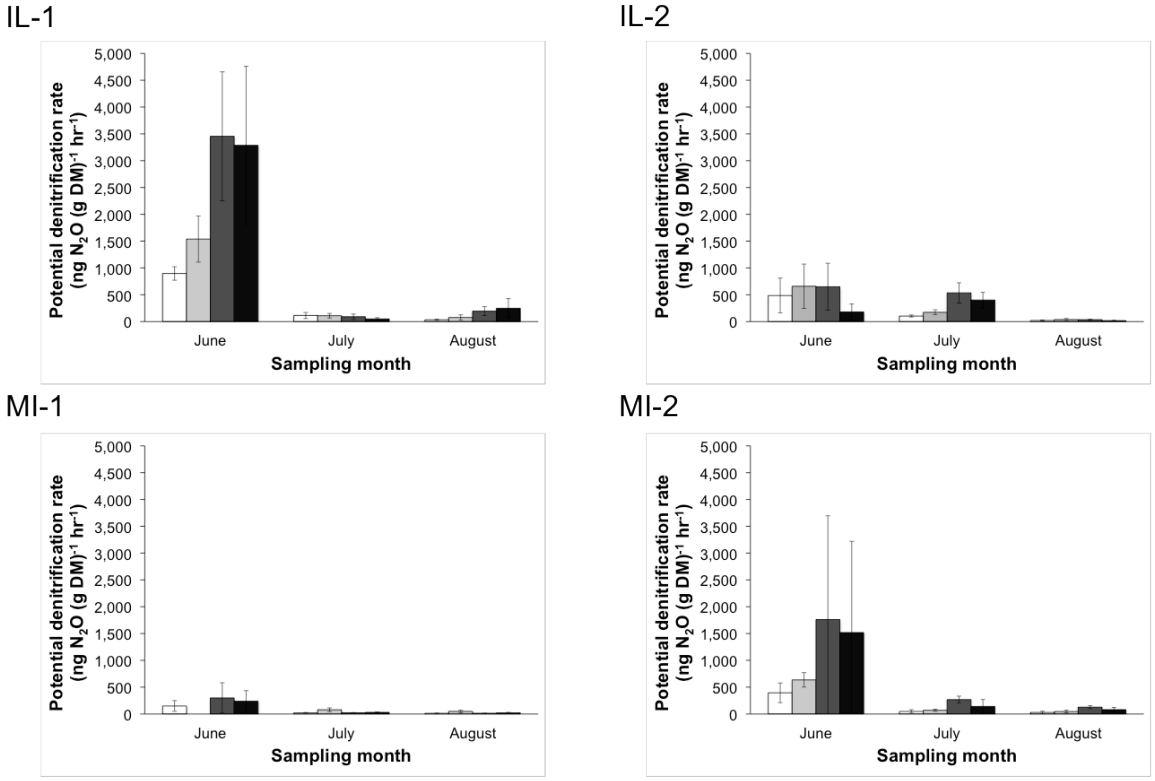


Figure 4.1. Summary of potential denitrification rates (ng N_2O (g DM)$^{-1}$ hr^{-1}) (mean ± SD) along the upland to wetland gradient (plots A – D) by month. Bars are colored white to black to represent average rate along an upland to wetland gradient. Microbial activity was averaged across transects along the gradient for each sampling month at each wetland site: IL-1, IL-2, MI-1 and MI-2.

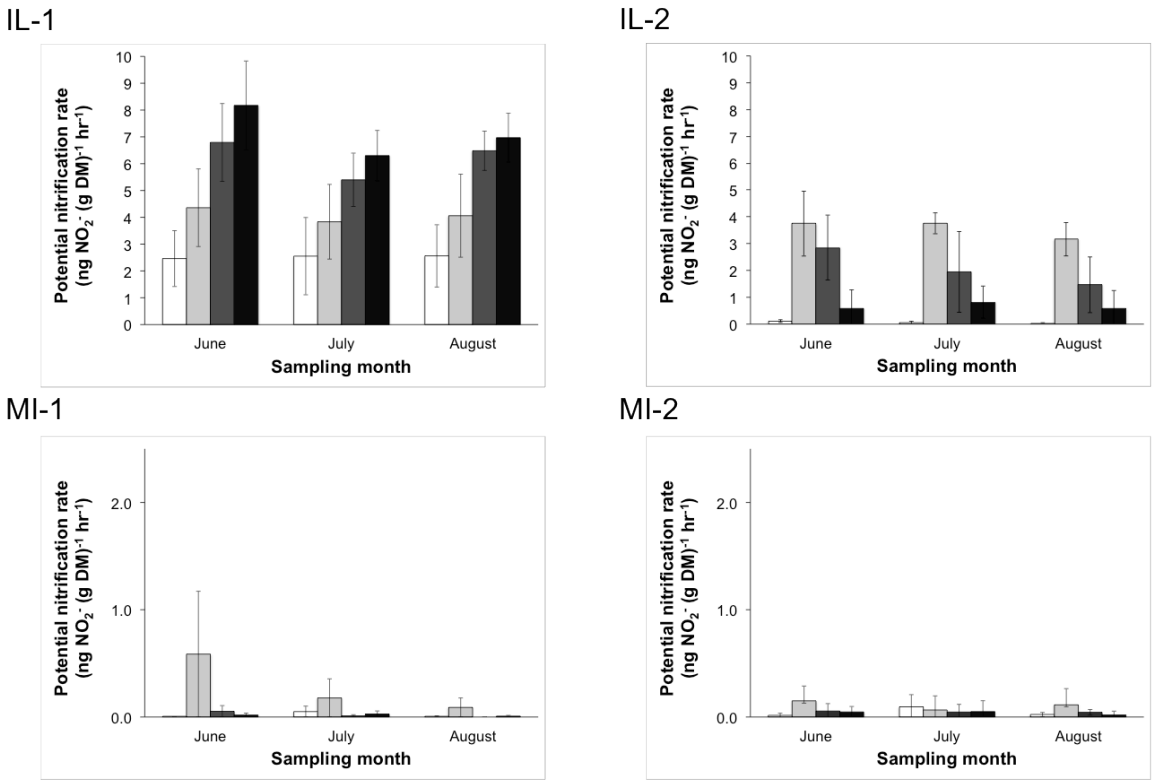


Figure 4.2. Summary of potential nitrification (ng NO_2^- (g DM)$^{-1}$ hr^{-1}) (mean ± SD) along the upland to wetland gradient (plots A – D) by month. Bars are colored white to black to represent average rate along an upland to wetland gradient. Microbial activity was averaged across transects along the gradient for each sampling month at each wetland site: IL-1, IL-2, MI-1, and MI-2.

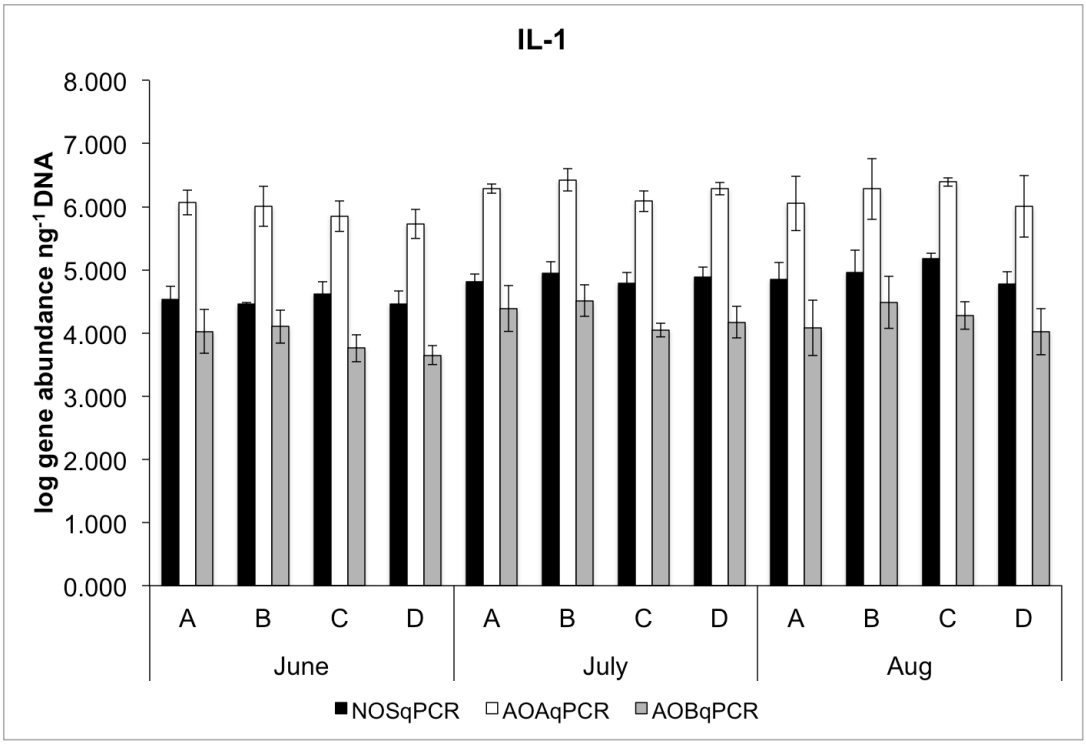
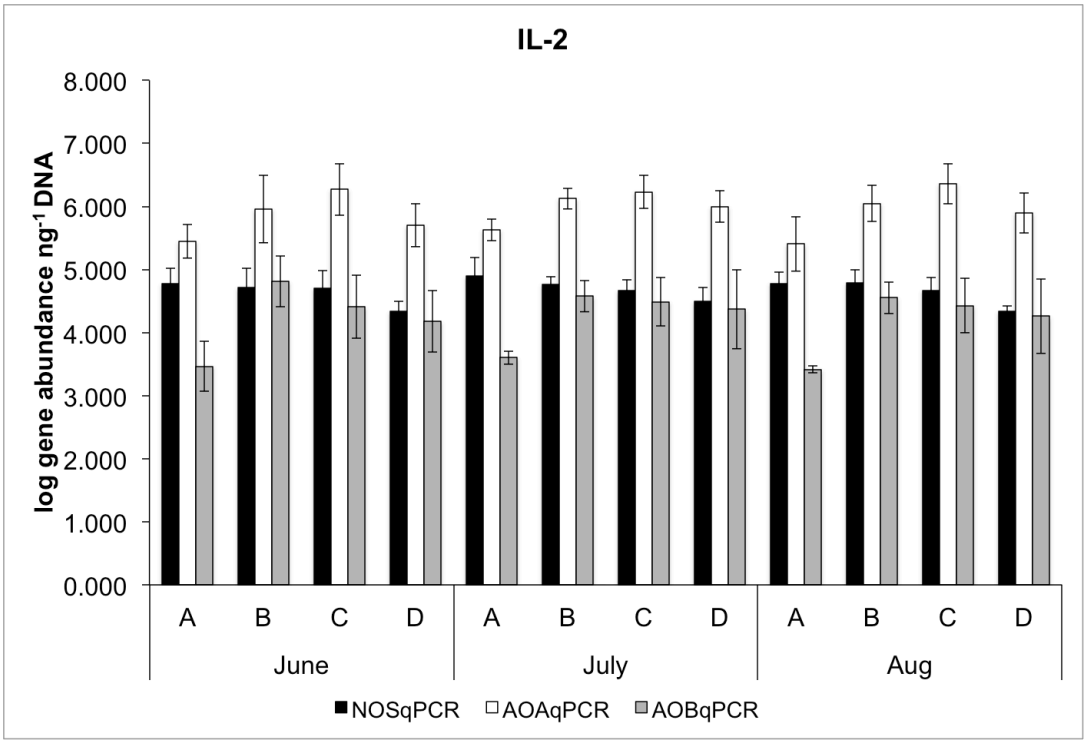


Figure 4.3. Summary of *nosZ* (black bars), archaeal *amoA* (white bars) and bacterial *amoA* (gray bars) gene abundance based on quantitative PCR along the upland to wetland gradient (plots A – D). Gene abundance was averaged across transects along the gradient for each sampling month at each wetland site: IL-1, IL-2, MI-1, and MI-2. Missing bars represent gene abundance observed below detection limits.

Figure 4.3 (cont.)



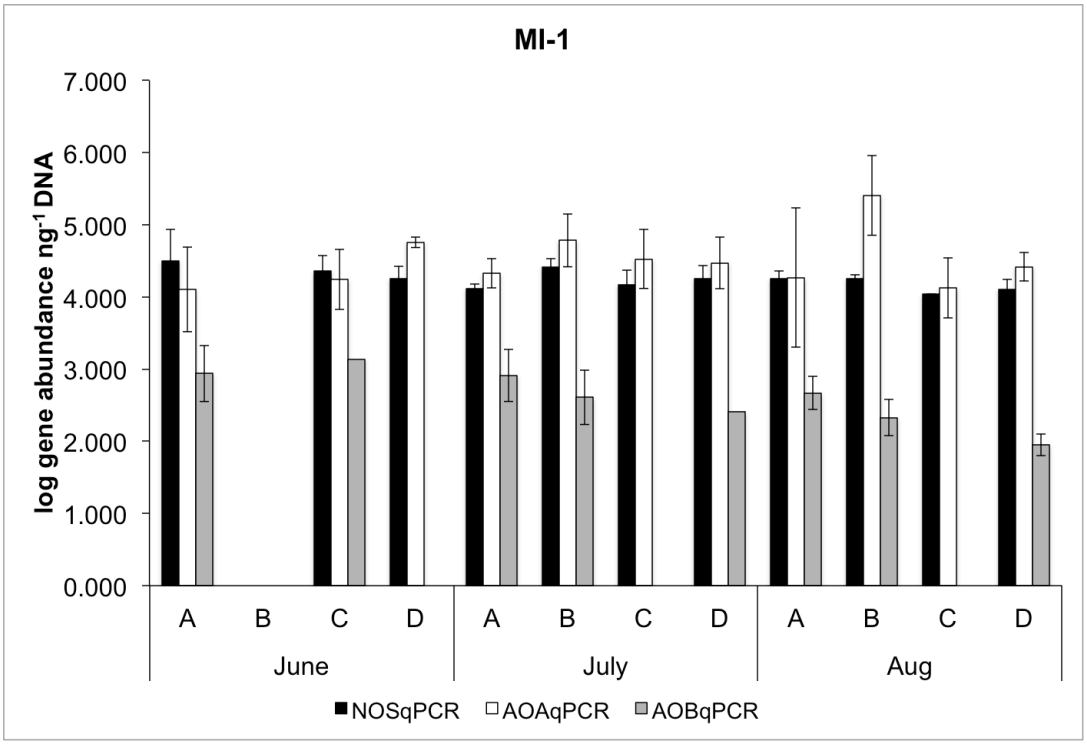
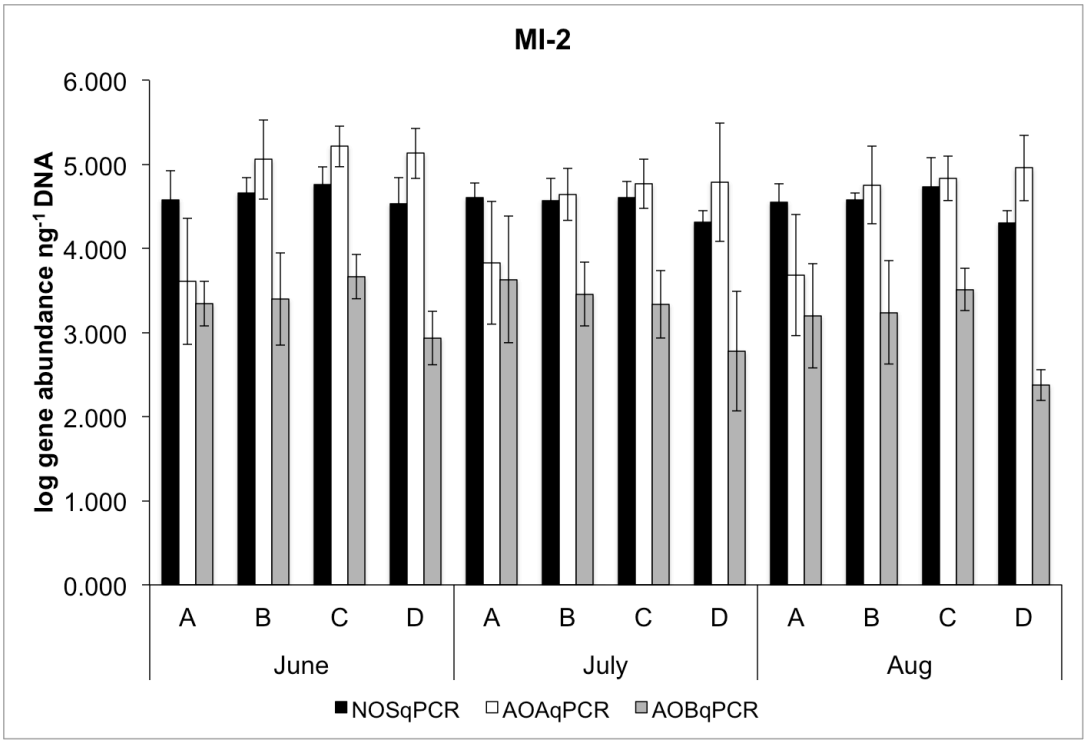


Figure 4.3 (cont.)



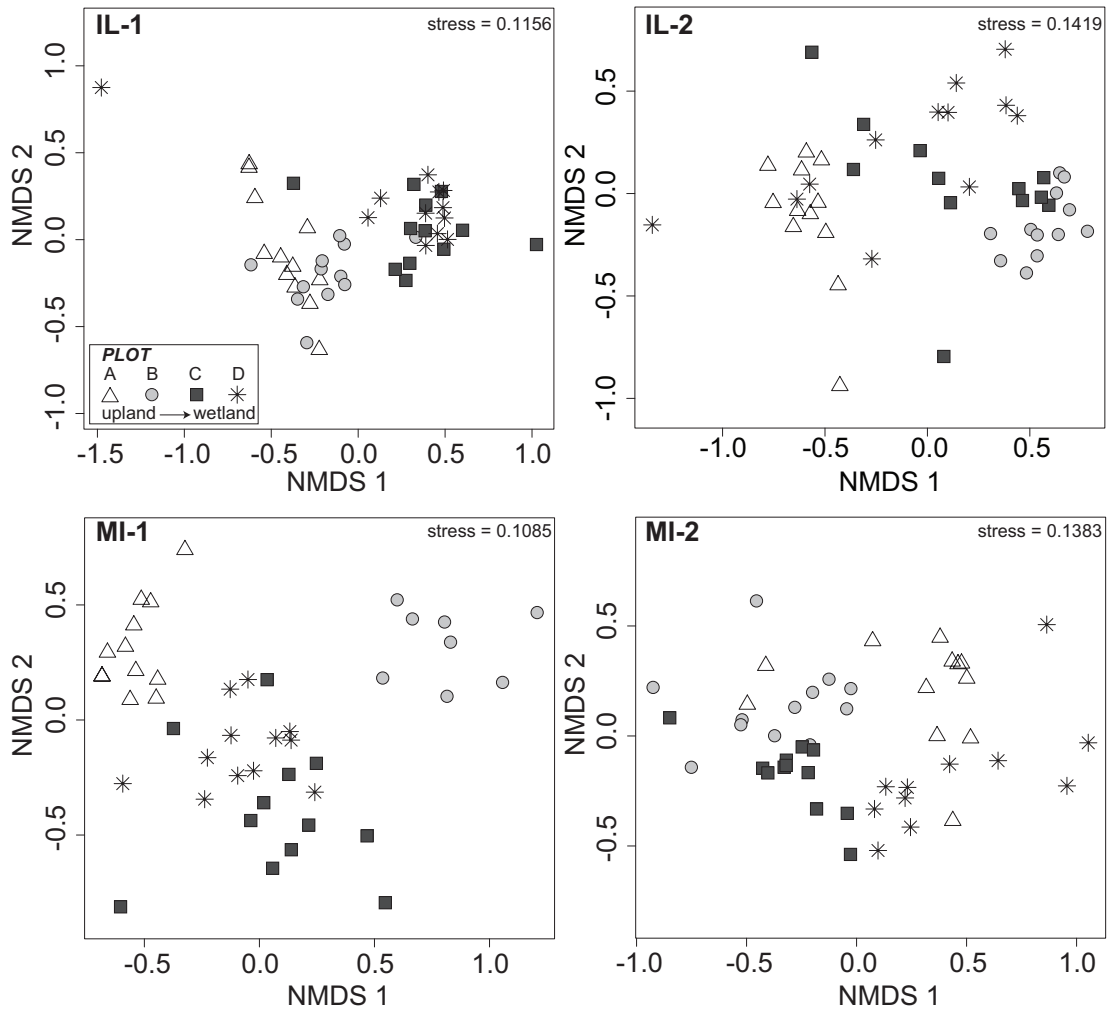


Figure 4.4. Non-metric multidimensional scaling plot of denitrifier community composition. Symbols are colored white to black and differ in shape to represent samples along an upland to wetland gradient. Each point represents the community composition of the denitrifiers based on *nosZ* T-RFLP relative fluorescence at different wetland sites: IL-1, IL-2, MI-1, and MI-2.

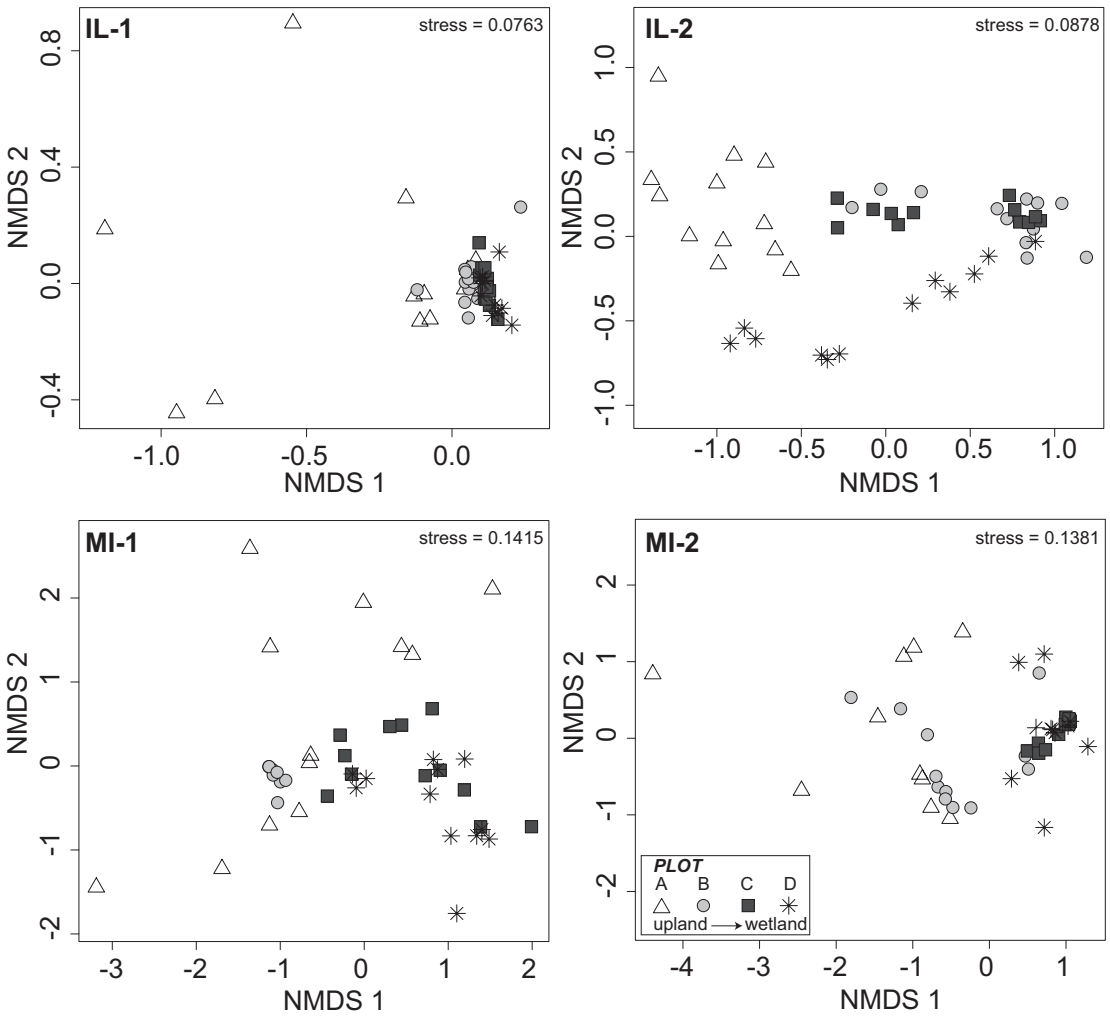


Figure 4.5. Non-metric multidimensional scaling plot of ammonia oxidizer community composition. Symbols are colored white to black and differ in shape to represent samples along an upland to wetland gradient. Each point represents the community composition of the ammonia oxidizers based on archaeal *amoA* T-RFLP relative fluorescence at different wetland sites: IL-1, IL-2, MI-1, and MI-2.

CHAPTER 5

HYDROLOGIC HISTORY CONSTRAINS WETLAND MICROBIAL STRUCTURE AND FUNCTION UNDER EXPERIMENTAL DRYING/WETTING REGIMES

ABSTRACT

Understanding how hydrologic history impacts microbial communities and their response to environmental change can inform management of wetland nutrient cycling functions. Recent experiments have shown that distinct microbial communities from contrasting environmental histories are also functionally different, and when these communities are exposed to the same contemporary environmental conditions, microbial function shifts to different degrees. To understand the degree to which history can constrain function of microbial communities, it is necessary to carry out controlled laboratory experiments. The objective of this study was to compare the response of microbial communities developed under different hydrologic regimes (upland vs. wetland) to varying moisture treatments (dry, wet-dry, saturated) in an experimental mesocosm. Local soil factors (e.g., soil moisture, inorganic nitrogen), and microbial community composition, abundance, and activity of denitrifiers and ammonia oxidizers were assessed. Wetland soils were characterized by higher soil fertility, pH and moisture levels, and also supported high potential denitrification and nitrification rates compared to upland soils. Hydrologic history*treatment effect significantly contributed to group differences in microbial activity (ANOVA: denitrification – $F_{(2,17)} = 20.78$, $P < 0.0001$; nitrification – $F_{(2,17)} = 12.30$, $P = 0.0005$), while the main effect of hydrology history contributed to group differences in community composition (PERMANOVA: denitrifier – $R^2 = 0.6722$, $P = 0.001$; archaeal ammonia oxidizer, $R^2 = 0.3816$, $P = 0.001$), and microbial gene abundance (ANOVA: denitrifier

– $F_{(1,18)} = 21.47$, $P = 0.0002$; archaeal ammonia oxidizer – $F_{(1,18)} = 78.72$, $P < 0.0001$; bacterial ammonia oxidizer – $F_{(1,18)} = 10.41$, P 0.0050). In response to the drying/flooding treatments, evidence of denitrifier, but not ammonia oxidizer community change was observed; and denitrifier and ammonia oxidizer gene abundance was higher in upland than wetland soils. Potential denitrification rate significantly increased under wetter conditions, whereas potential nitrification rates did not significantly change following drying/flooding treatments in many cases. Significant shifts in denitrification, but not nitrification, provide evidence that short-term fluctuations in soil moisture are expected to influence denitrification rates to a higher extent than nitrification rates at this wetland. Variation in microbial functional response can result in a shift in dominant nitrogen cycling transformations within a wetland as a consequence of microorganisms responding differently to environmental change.

INTRODUCTION

Linking community structure and ecosystem function is important for understanding ecosystem-level consequences of environmental change. Composition, abundance, and activity of individuals within specific functional guilds dictate ecosystem processes, where diversity within functional guilds is often more predictive of ecosystem processes than species richness (Hooper and Vitousek 1997, Naeem and Wright 2003, Tilman et al. 1997). The community structure-ecosystem function relationship can change in response to environmental conditions, making it necessary to understand interactive effects between functional guild composition and the environment on ecosystem processes (Cardinale et al. 2000).

Microbial systems are ideal for addressing relationships between community structure and ecosystem function (Jessup et al. 2004). Microorganisms produce enzymes that catalyze

ecologically relevant biogeochemical transformations (e.g., denitrification, nitrification) (Zak et al. 2006). The presence of genes encoding these enzymes can be used to define microbial functional guilds. Guilds can be studied by analyzing the diversity, composition, and abundance of genes responsible for specific biogeochemical transformations. The composition of microorganisms with a variety of physiological responses within a functional guild determines how environmental conditions influence ecosystem processes. If a community is composed of functionally redundant taxa, loss of certain taxa may be compensated for by other taxa within the community resulting in similar process rates. In contrast, if communities contain taxa that make unique contributions to function, loss of specific taxa will directly impact ecosystem processes (Naeem et al. 2002).

Microbial communities that develop under different conditions can variably respond to environmental factors. Communities are composed of microorganisms that range in their tolerance to local environmental conditions (Cavigelli and Robertson 2000, Strickland et al. 2009). The legacy of prior land use can result in permanent changes in community structure and function, where microbial response to the current environment is constrained by the existing assemblage (Fraterrigo et al. 2006, Strickland et al. 2009). For example, soil factors such as moisture, redox condition, and nutrient availability change in response to hydrologic manipulation within restored wetlands. This heterogeneous environment can provide different microhabitats for microorganisms (Mentzer et al. 2006, Schimel et al. 2007). Various microbial taxa can exploit particular conditions of fluctuating hydrology, where recharge of nutrients and oxygen occurs dynamically (Keddy 2000), while other taxa may be more successful at persisting in more stable conditions that support anoxic, nutrient-limited microhabitats (Bossio and Scow 1998, Pett-Ridge and Firestone 2005). Microbes capable of tolerating a range in redox conditions

persist under more dynamic hydrology than microbes that are more sensitive and restricted in metabolic processes (Pett-Ridge and Firestone 2005, Schimel et al. 2007, DeAngelis et al. 2010). Differential microbial response to hydrologic conditions directly affects microbially-mediated ecosystem functions in potentially predictable ways. Therefore, experiments under controlled conditions are needed to evaluate how contemporary environmental drivers interact with and influence microbial structure-function relationship (Reed and Martiny 2007).

Examining microorganisms involved in nitrogen (N) cycling transformations can provide insight into structure-function relationships, and the majority of N cycling transformations are microbially-mediated and well characterized (Francis et al. 2007, Wallenstein et al. 2006). Nitrate (NO_3^-) removal functions of ecosystems occur via the denitrification pathway ($NO_3^- \rightarrow N_2$), while internal N cycling can occur through nitrification ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$). Both of these transformations are particularly sensitive to oxygen concentrations, which is often controlled by moisture in the soil. Microbial functional groups capable of carrying out denitrification and nitrification are also differentially influenced by other local environmental factors such as soil pH, temperature, soil inorganic N and organic matter content (Cavigelli and Robertson 2000, Nicol et al. 2008, Prosser 2007, Sirivedhin and Gray 2006, Van Cleemput et al. 2007, Wertz et al. 2007). Physiological differences among and within microbial functional groups can result in varying degrees of tolerance to local soil conditions such as oxygen/soil moisture levels in the environment (Jayakumar et al. 2009). Investigating the influence of oxygen availability on microorganisms due to changes in soil moisture is important for understanding links between microbial structure and nitrogen cycling processes (Francis et al. 2007, Kowalchuk and Stephen 2001, Wallenstein et al. 2006).

From previous chapters of this dissertation, microbial community composition and activity related to denitrification and nitrification significantly differed between upland and wetland soils, and microbial activity decreased, while community composition remained relatively similar over the short-term (i.e., months). The objective of this study was to investigate the response of microbial communities shaped by different historic hydrologic regimes (upland vs. wetland) to experimental drying-flooding treatments (dry, wet-dry, saturated). The structure and function of populations involved in specific N transformations (e.g., denitrifiers, ammonia oxidizers) and their capacity to respond to flooding-drying manipulations were examined. Structure was represented by microbial community composition based on microbial molecular analysis (terminal restriction length polymorphism – T-RFLP and quantitative PCR based upon diagnostic genes for each functional group). Function was assessed by measuring potential denitrification and nitrification assays. It was predicted that the distinct hydrologic history of the upland and wetland soils would influence the initial microbial assemblages, and that hydrologic history would constrain microbial community composition and activity under different soil moisture treatments. Further, it was predicted that microbial functions sensitive to drying/flooding pulses would respond to soil moisture treatments, while microbial community composition was predicted to remain stable in the short-term. Therefore, hydrologic history was expected to influence the range of responses to flooding disturbance treatments by structuring the microbial community as either tolerant or intolerant of changes in drying/flooding conditions.

MATERIALS AND METHODS

Sample collection

The St. Joseph Wetland was under conventional agricultural management until ecological restoration began in 2006. The hydrology at St. Joseph Wetland fluctuates in response to precipitation and flooding by an adjacent river. For the experiment, soil was collected from plots established along an environmental gradient described in Chapter 4 on 30 July 2010. Soil samples were collected to about a 12 cm depth using a shovel at St. Joseph Wetland (40.12, -88.05). Two locations, upland and wetland were randomly sampled between 2 established transects, spanning 20 m. The upland and wetland sites represented different hydrologic histories. The upland plot receives floodwaters if the adjacent river overflows, but this area remains mostly dry throughout the season. The wetland site is more often inundated with water during early spring to early summer, and dries out during the hotter months. The difference in amount and length of inundation of water in this restored floodplain strongly structures microbial community composition and function (refer to Chapter 3).

Soils from upland and wetland plots, representing different hydrologic histories, were collected into sterilized plastic bins. Soils were stored in closed containers overnight at room temperature, until mesocosm preparation. Prior to mesocosm set-up, upland and wetland soils were processed separately. Soil was passed through a 1 in. sieve and homogenized. Three soil samples from upland and wetland hydrologic histories were transferred into sterile 15 mL tubes, and stored at -80 °C for molecular analysis.

Mesocosm set-up

A factorial treatment design was used to test the interaction of hydrologic history and drying/wetting disturbance in this wetland ecosystem. Two hydrologic histories (upland,

wetland) and 3 moisture levels (dry, wet-dry, wet) were established in four replicate groups. Soil from upland and wetland histories were used to fill 24 standard 6 in pots (5.6 in deep). Volumetric moisture content was recorded to capture initial moisture conditions in the pots. Volumetric water content averaged about 8% in the upland soils and about 38% in the wetland soils at the beginning of the experiment. To standardize all pots before applying flooding treatments, 200 mL of autoclaved, deionized water was added to each pot. On day 2, flooding treatments began. For each hydrologic history (upland or wetland), 3 drying/flooding treatments were applied: dry (no water added), wet-dry (200 mL water added every 3 days), and saturated (water added to about 2 in above soil). All pots were incubated in a greenhouse space for 2 weeks. We assumed that a 2-week incubation time was representative of a natural drying or flooding event within the restored wetland in order to provide an accurate assessment of short-term microbial response to the experimental manipulation. Indicator for reduction in soils (IRIS) tubes (InMass Technologies, West Lafayette, IN, USA) were incubated in one representative pot, for each history*flooding treatment. IRIS tubes were used to assess redox status over the two-week incubation to confirm that wet treatments supported reducing conditions (Castenson and Rabenhorst 2006, Jenkinson and Franzmeier 2006). Volumetric soil moisture was monitored using a HydroSense Soil Moisture System (Campbell Scientific, Australia) to non-destructively measure volumetric moisture content to a depth of 12 cm during the experiment.

After the two-week incubation, soils were collected from each pot using a hand trowel. Soil was sampled vertically from top to bottom of each pot and half of the soil was collected and homogenized (to account for any bias that developed from vertical stratification). Soil from each pot was transferred into 15 mL tubes and stored at -20 °C for molecular analysis.

Activity assays and chemical analyses were completed for 3 replicates from upland and wetland hydrologic histories to represent the baseline conditions prior to the flooding treatment. Pre- and post-treatment soils were analyzed for gravimetric soil moisture, available nitrate and ammonium, and potential denitrification and nitrification rates. Soil organic matter and soil pH were analyzed according to previously described methods in Chapter 2**.**

Soil chemical analyses

For each set of baseline and post-treatment samples, gravimetric soil moisture was determined on a 20-30 g subsample. Field moist soil was dried overnight at 105 ºC and moisture content was calculated from the proportion of water weight to oven-dried soil weight. To assess inorganic N concentration, about 5 g of field moist soil was extracted with 2 M KCl, and available ammonium (NH_4^+-N) and nitrate (NO_3^--N) were analyzed in the extracts. Analysis was based on colorimetric analyses using an auto analyzer (Lachat Instruments/Hach Company, Loveland, CO).

Samples previously collected at upland and wetland locations of St. Joseph Wetland in June 2010 were analyzed for soil organic matter content. A subsample of air-dried soil was finely ground and analyzed to for total organic matter (total organic C and total N) based on combustion methods (ECS 4010, COSTECH Analytical Instruments, Valencia, CA, USA). Soil pH was determined for a 5 g sample based on a 1:1 soil:water ratio.

Potential denitrification assay

On fresh soil samples, denitrification potential of the soil microbial community was estimated using the acetylene inhibition method (Royer et al. 2004). In 125-mL Wheaton bottles, 90 mL 2mM KNO_3 solution and 1.3 mL of chloramphenicol (100 mg ml^{-1}), and about 25 g of soil were combined. Bottles were sealed with septa-centered caps, shaken, purged with He for 5

min. Bottles were vented prior to starting the assay. Before gas samples were collected, each bottle was shaken for 5 minutes to equilibrate N_2O in aqueous and sediment phases. Gas samples (15 mL) were collected at 0, 1, 2, and 3 hours.

Gas samples were analyzed for N_2O using a Shimadzu 2014 greenhouse gas analyzer (Shimadzu Scientific Instruments, Columbia, MD). Gas standards ranging from 0.1 ppm-v to 7.46 ppm-v N_2O were generated from 99% N_2O (Grace Divisions, Deerfield, IL). The N_2O concentrations of each sample per unit dry mass were plotted against sampling time, and the slope of this line was measured as the potential denitrification rate (ng N_2O g^{-1} dry mass hr^{-1}). For the majority of samples, N_2O production from each sample was linear during the assay. To remain within the range of N_2O standards, all gas samples were diluted prior to GC analysis in order for N_2O concentrations to remain within the range of standards. Conversion of field-moist soil weight to oven-dry weight was based on gravimetric soil moisture for each sample.

Potential nitrification assay

Potential nitrification activity of the soil microbial community was assayed using a 5-hour incubation (Kandeler 1996). Approximately 5 g aliquots of field-most soil were weighed into two replicate 125-mL flasks. In another bottle, another 5 g of soil was weighed for the control from each sample. Prior to incubation, 20 mL of 1 mM $(NH_4)_2SO_4$ and 0.1 mL of 2 M $NaClO_3$ were added to prepared soils. The replicates were shaken for approximately 5 hours at 130 rpm. The filtrate from the 5-hour incubation and for control samples stored at -20°C was collected and analyzed for NO_2^- produced. Analysis of NO_2^--N was based on a colorimetric assay (Kandeler 1996). Calibration standards range from 0, 0.2, 0.4, 0.8, to 1 μg NO_2^--N mL^{-1}. Potential nitrification rate was calculated as the difference between control and incubated soil per mass of soil per hour. Each potential rate measurement was the average of two replicates.

DNA extraction and purification

Extraction of total genomic DNA using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH) was carried out on freeze-dried soil samples. To remove contaminating humic acids, genomic DNA was purified using a cetyl trimethyl ammonium bromide (CTAB) extraction (Sambrook and Russell 2001). DNA concentration was adjusted to a standard concentration of 20 ng μl^{-1} prior to molecular analyses.

Microbial community analyses

Composition of denitrifying microorganisms was assessed using terminal restriction fragment length polymorphism (T-RFLP) of the *nosZ* gene. In this study, "denitrifier taxa" specifically refers to the subset of microorganisms that carry out the last step of denitrification ($N_2O \rightarrow N_2$) catalyzed by nitrous oxide reductase. PCR reactions were used to amplify the *nosZ* gene, which encodes the catalytic subunit of nitrous oxidize reductase (Rich et al. 2003). PCR reactions contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of *Taq* polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 µl. The *nosZ* gene was amplified using *nosZ*-F-1181, 5'-CGCTGTTCITCGACAGYCAG-3' and *nosZ*-R-1880, 5'-ATGTGCAKIGCRTGGCAGAA-3' to yield a 700 bp PCR product (Rich et al. 2003). The *nosZ* reverse primer was labeled with the phosphoramidite dye 6-FAM. Reactions were cycled with initial denaturation at 94 °C for 3 min, followed by 25 cycles of 94 °C for 45 s, 56 °C for 1 min, and 72 °C for 2 min, with a final extension carried out at 72 °C for 7 min. PCR product from two 50 µl reactions were combined and concentrated using the Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA). Amplicons generated from each sample were digested with *Alu*I and *Hha*I in single-enzyme restriction digests.

Community composition of ammonia oxidizing archaea was assessed using T-RFLP analysis. The "ammonia oxidizer taxa" specifically refers to microorganisms that carry out the rate-limiting, first step of nitrification, catalyzed by ammonia monooxygenase (Kowalchuk and Stephen 2001). The *amoA* gene encodes the catalytic α-subunit of archaeal ammonia monooxygenase. PCR reactions contained 50 mM Tris (pH 8.0), 250 µg of bovine serum albumin per ml, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 20 pmol of each primer, 2.5 U of Taq polymerase (Promega, Madison, WI), and 100 ng of extracted DNA in a final volume of 50 µl. The *amoA* gene was amplified using *amoA-F*, 5'-STAATGGTCTGGCTTAGACG-3' and *amoA-R*, 5'-GCGGCCATCCATCTGTATGT-3' to yield a 650 bp amplicon (Francis et al. 2005). The *amoA* forward primer was labeled with the fluorescent dye HEX, the *amoA* reverse primer was labeled with the fluorescent dye NED. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 45 s, 53 °C for 1 min, and 72 °C for 1 min, with a final extension carried out at 72 °C for 15 min. PCR product from a 50 µl PCR reaction was concentrated using the Qiagen MinElute PCR purification kit (Qiagen, Valencia, CA, USA). Amplicons from each sample were digested with *Rsa*I, yielding 2 fluorescently-labeled terminal restriction fragments (T-RFs).

The length and relative abundance of terminal restriction fragments (T-RFs) were determined by denaturing capillary electrophoresis using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Electrophoresis conditions were 63 °C and 15 kV with a run time of 120 minutes using POP-7 polymer. The internal size standard for the *nosZ* and *amoA* T-RFLP analysis was the ABI GeneScan ROX 1000 size standard (Applied Biosystems, Foster City, CA). GeneMarker version 1.95 (SoftGenetics, State College, PA) was used for size-calling T-RFs. For our analyses, each terminal restriction fragment represents a microbial taxon, and the

combination of all T-RFs produced from a sample corresponds to the assemblage of microorganisms present in a soil sample. Each T-RF was expressed as a proportion of the observed community by normalizing the signal strength (i.e., peak area) of each T-RF peak to the total fluorescence observed for each digest to account for run-to-run variations in signal detection (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Terminal restriction fragments above a detection threshold of 100 relative fluorescence units for *nosZ* T-RFs and 300 relative fluorescence units for *amoA* T-RFs were included in community analyses. Normalized T-RFLP profiles produced from separate digests of each PCR product (or from different fluors, in the case of *amoA*) were concatenated prior to statistical analysis (Fierer and Schimel 2003, Peralta et al. 2010).

Denitrifier and ammonia oxidizer gene abundance

Quantitative PCR of *nosZ,* archaeal and bacterial *amoA* was carried out in triplicate in 10 ul reactions. The *nosZ* gene was amplified using Nos1527F, 5'- CGC TGT TC(A/C/T) TCG ACA G(C/T)C A-3' (Kloos et al. 2001) and nosZ1622R, 5'- CGC (G/A)A(C/G) GGC AA(G/C) AAG GT(G/C) CG-3' (Throback et al. 2004). The archaeal *amoA* gene was amplified using PCR primers Arch-amoAF (5'-STAATGGTCTGGCTTAGACG-3') and Arch-amoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al. 2005); and the bacterial *amoA* gene was amplified using PCR primers amoA-1F; 5-GGGG TTTCTACTGGTGGT-3' and amoA-2R; 5-CCCCTCKGSAAAGCCTTCTTC-3' (Rotthauwe et al. 1997). PCR reactions contained 1X SYBR green master mix (Applied Biosystems Inc., Foster City, CA), 0.4 μM of each primer, 0.5 μg μl^{-1} bovine serum albumin, and 2 ul of soil DNA of known concentration. Fragments were amplified with an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of 95°C for 1 min, 56 °C for 1 min and 72 °C for 1 min. Standard curves were obtained based on serial

dilutions of mixed PCR product from wetland soil samples. Reactions were analyzed on a 384-well Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems Inc., Foster City, CA).

Statistical analyses

Analysis of variance (ANOVA) of the main effects (hydrologic and drying/wetting treatment) as well as interactions of the main effects was carried out on differences in potential denitrification and nitrification rates as well as for denitrifier and ammonia oxidizer gene abundance (change in rate from pre- to post-drying/flooding treatment). Hydrologic history and drying/flooding treatment were considered fixed factors, and replicate was a random factor. Tukey's HSD test was carried out to determine between-group differences in potential denitrification and nitrification rates. ANOVA was run in the MIXED procedure of SAS (PROC MIXED, SAS v9.2, SAS Institute).

Venn diagrams were used to visualize the overlap of individual denitrifier and ammonia oxidizer taxa between upland and wetland soils. Venn diagrams were produced (and proportioned according to total number of taxa represented) using the Venn Diagram Plotter developed at Pacific Northwest National Laboratories (software found at http:omics.pnl.gov). A taxon was included in the underlying presence-absence matrix if it was observed in at least two of four of the replicate pots within a drying/flooding treatment.

Bray-Curtis similarity matrices were generated for denitrifier and ammonia oxidizer communities (Legendre and Legendre 1998). Terminal restriction fragment (T-RF) relative fluorescence data was treated as relative abundance data in our statistical procedures, as is common in microbial ecology studies (Kent et al. 2007, Rees et al. 2004, Yannarell and Triplett 2005). Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship

among microbial communities in baseline pre- and post-drying/flooding treatment. NMDS is based on the rank order relation of dissimilarities using the Bray-Curtis similarity coefficient, where the largest distance between points on the ordination plot represents the most dissimilar microbial communities. NMDS were carried out using PRIMER version 6 (PRIMER-E Ltd, Plymouth, UK). Permutational (nonparametric) multivariate analysis of variance (PERMANOVA) using the adonis function in the R Statistics Package (R Development Core Team 2010) was used to evaluate the influence of hydrologic history and drying/flooding treatment on denitrifier and ammonia oxidizer community composition (McArdle and Anderson 2001).

RESULTS

Soil characteristics associated with hydrologic history and drying/flooding treatment

Our experimental manipulations of drying/flooding induced reducing conditions in saturated pots and less reduced conditions in dry treatments. The most reduced conditions were observed in the wet treatment, where both upland and wetland soils were inundated with water (Fig. 5.1). In general, wetland soils supported higher potential denitrification and nitrification rates than upland soils. Higher total soil organic C, total N, C:N ratio, soil moisture, and pH were observed in in the baseline wetland soils (Table 5.1). Inorganic nitrogen levels changed across the experimental treatments within each soil type, where nitrate concentration decreased and ammonium increased from dry to wet treatments, respectively (Table 5.2).

A significant interaction between hydrologic history and drying/flooding treatment was detected for potential denitrification rate (ANOVA, history*treatment: $F_{(2,18)} = 20.78$, $P < 0.0001$) (Fig. 5.2a, Table 5.3a). Differences in potential denitrification rates (between baseline

and post-treatment) were significantly higher for wet-dry and wet treatments (Tukey HSD, $P_{adj} < 0.05$) (Fig. 5.2a, Table D.1). Potential denitrification rates were significantly different between the following comparisons (hydrologic history-drying/flooding treatment): upland-dry vs. wetland-dry, upland wet/dry-wet vs. upland-wet, upland-wet vs. wetland-wet, and between wetland-wet/dry vs. wetland-wet treatments (Tukey HSD, $P_{adj} > 0.05$) based on *posthoc* comparisons (Fig. 5.2a, Table D.1).

A significant interaction between hydrologic history and drying/flooding treatment was detected for potential nitrification rates (ANOVA, history*treatment: $F_{(2,18)} = 12.30$, $P = 0.0004$) (Fig. 5.2b, Table 5.3b). However, potential nitrification rate was similar between upland and wetland hydrologic histories (ANOVA, history: $F_{(1,18)} = 4.05$, $P = 0.0595$) (Fig. 5.2, Table 5.3b). Based on *post-hoc* contrasts, significant differences were detected between dry and wet-dry (Tukey HSD, $P_{adj} = 0.0028$) and dry and wet treatments within wetland soils (Tukey HSD, $P_{adj} = 0.0017$) (Fig. 5.2, Table D.1). In addition, potential nitrification rates were higher in upland vs. wetland soils experiencing continuously dry conditions (Tukey HSD, $P_{adj} = 0.0010$). Nitrification rates were higher in upland soils receiving the alternating wet-dry treatment compared to wetland soils receiving the continuously dry treatment, perhaps due to increased inorganic N availability (Tukey HSD, $P_{adj} = 0.0021$) (Fig. 5.2, Table D.1).

Response of microbial community composition and activity to drying/flooding treatment

At the beginning of the experiment, about 14% of taxa overlap among denitrifiers was shared between upland and wetland baseline soils compared to about 58% for ammonia oxidizer taxa (Fig. 5.3). However, the number of shared taxa was higher among denitrifiers than ammonia oxidizer taxa between drying/flooding treatments (data not shown).

A total of 55 unique *nosZ* terminal restriction fragments (T-RFs) were detected in denitrifying communities. Denitrifier community composition varied by hydrologic history, resulting in distinct communities in upland and wetland soils (PERMANOVA, $R^2 = 0.6722$, $P = 0.0010$) (Fig. 5.4a, Table 5.3a). The drying/flooding treatment contributed a significant, but relatively small, proportion of variation in denitrifier community composition (PERMANOVA, $R^2 = 0.0399$, $P = 0.0390$) (Fig. 5.4b, Table 5.3a). Higher denitrification rates were associated with denitrifier communities originating from wetland soil and under upland conditions subjected to wet-dry and wet treatment conditions (Fig. 5.5a). Denitrifier communities carrying out higher potential denitrification rates were also associated with a lower nitrate-N concentration in the experimentally saturated soils (Fig. 5.5b).

A total of 42 unique *amoA* T-RFs were detected in ammonia oxidizing communities pre- and post-drying/flooding treatment. Ammonia oxidizer community composition varied by hydrologic history; different communities developed under original upland and wetland conditions (PERMANOVA, $R^2 = 0.3816$, $P = 0.0010$) (Fig. 5.6a, Table 5.4b). Drying/flooding treatment did not significantly influence variation in ammonia oxidizer community composition (PERMANOVA, $R^2 = 0.0315$, $P = 0.5385$) (Fig. 5.6b, Tables 5.4b). Higher potential nitrification rates were associated with distinct ammonia oxidizer community composition in wetland soils in addition to detectable differences in activity in response to drying/flooding treatments (Fig. 5.7a, Table D.1). Lower ammonium levels were associated with communities in the experimentally flooded pots originating from upland and wetland soils (Fig. 5.7b).

Response of denitrifier and ammonia oxidizer gene abundance

Denitrifier (*nosZ*), archaeal and bacterial ammonia oxidizer (AOA, AOB) gene abundance significantly differed between upland and wetland soils (ANOVA, *nosZ* qPCR:

history: $F_{(1,18)} = 21.47$, $P = 0.0002$; AOA qPCR: history: $F_{(1,18)} = 78.72$, $P < 0.0001$; AOB qPCR: history: $F_{(1,18)} = 10.41$, $P = 0.0047$) post-drying/flooding treatment (Fig. 5.8, Table 5.5, Table D.2). No significant differences in changes in gene abundance were observed among post-drying/flooding treatments (Table 5.5). In addition, changes in gene abundance were not observed among post-drying treatments and change in gene abundance was not significantly correlated to microbial activity (*nosZ*/denitrification: r = -0.2326, $P = 0.2740$; AOA/nitrification: r = 0.2447, $P = 0.2492$; AOB/nitrification: r = -0.0408, $P = 0.8499$).

DISCUSSION

The interactive effects between functional guild composition and the environment, and their consequences for ecosystem processes were examined in this study. The community structure-function link was examined by tracking denitrifier and ammonia oxidizer, community composition, abundance, and activity between soils developed under varying hydrologic histories. We investigated whether hydrologic history constrained the response of microbial communities in each soil to drying/flooding treatments. Microbial transformations could be influenced by flooding/drying events, which warrants investigation of how the ecosystem service of denitrification responsible for nitrate removal could potential offset by nitrate inputs via nitrification. Our experimental manipulation of drying/flooding conditions allowed us to examine the influence of changing moisture regimes on microbial processes related to N release and retention in wetlands.

The initial conditions in which denitrifiers and ammonia oxidizers developed constrained microbial activity in response to drying/flooding treatments. Only small changes in denitrifier community composition and no change in ammonia oxidizer community composition after the

drying/flooding treatment were observed in this study. Potential denitrification rates were generally lower in upland soils, even under wet soil conditions, suggesting that despite saturated conditions, denitrifier communities in upland soils may be inhibited by historical hydrologic conditions. Higher nitrate levels in wetland soil may be supporting higher rates of denitrifier carried out by distinct denitrifier assemblages. Upland denitrifier taxa adapted to consistently aerobic environments may vary in denitrification rates compared to wetland denitrifier taxa. Denitrifier assemblages that developed under wetland conditions would have experienced more dynamic redox conditions (changed from saturated to dried conditions within a season) historically (based on *in situ* redox measurements, data not shown). These taxa must be able to exploit changing redox conditions and be poised to respond to changes in hydrology compared to upland microbial communities. These results were similar to those observed in a previous study by DeAngelis et al. (2010), where unique soil microbial communities adapted to fluctuating compared to more stable redox conditions. In a previous study, bacteria were not only able to persist under fluctuating redox conditions in tropical soils were adapted to dynamic redox regimes, but they were likely maintained by a range of physiological tolerance mechanisms (Pett-Ridge and Firestone 2005). These community differences have profound ecosystem function implications, for example, Mentzer et al. (2006) reported an increase in microbial activity in response to periodic flooding, which potentially contributed to altered nitrogen cycling rates.

In this wetland, potential nitrification rates were not as sensitive to changes in drying/flooding conditions compared to denitrification activity. It is possible that even under saturated conditions, microaerophilic habitats supported nitrification activity to some degree, and ammonia oxidizers in these soils are primed to carry out nitrification even under short-term

flooding conditions (Prosser 2007). It is also possible that nitrification was supported in soils characterized by relatively high ammonium (NH_4^+) levels and alkaline soil conditions (soil pH ranged from 7-8 at our site). Above around pH 6.5, NH_3 is dominant and is available to enter the cell more easily via diffusion. However, below about pH 6.5, NH_3 is ionized to NH_4^+, requiring active transport for NH_4^+ uptake (Prosser 2007) resulting in more limiting conditions. Overall, nitrification potential in our wetland is supported under the alkaline soil conditions and relatively high nitrogen loads in these wetland soils.

At this restored wetland, distinct denitrifier and ammonia oxidizer communities developed under contrasting soil conditions. Upland soils were characterized as relatively drier and less fertile than wetland soils. Different denitrifier and ammonia oxidizer assemblages were supported in the distinct habitats. Higher potential denitrification activities were observed in wetland compared to upland soils, whereas a more subtle change in nitrification activity was observed post-treatment. These results suggest ecosystem-level implications that can be revealed. If denitrification is more sensitive to drying/flooding events than nitrification, then production of nitrate from nitrification may result in loss of nitrogen through denitrification under anaerobic conditions. However, increased opportunity for nitrate leaching may occur under drier, aerobic conditions. Assimilative nitrate reduction can contribute to reducing nitrate loads, however nitrate reduction removes nitrate to a lesser degree than denitrification (Madigan and Martinko 2006). If denitrification is more sensitive to hydrologic disturbances, then dry periods may have the potential to contribute to nitrate loading if floodwaters quickly recede within a wetland.

Microbial communities are composed of taxa with a range of physiological capabilities, and these taxa can also vary in functional response to environmental changes or disturbances.

The diversity of microorganisms have been followed by the assumption that microbes are functionally redundant and that measuring function is sufficient for characterizing microbial functional response (Zak et al. 2006). However, not all microbes respond to environmental change similarly, and the present study indicates that environmental change can impart a range of microbial responses to temperature, carbon inputs (Balser and Wixon 2009, Strickland et al. 2009), and flooding/drying events. Differences in microbial functional response can be enhanced or constrained by hydrologic history, thereby resulting in ecosystem-level changes in function in the short-term and potential shifts in community composition in the long-term. This must then be considered in light of the varied response of microbial functions to contemporary drying/flooding events that can result in shifts in nitrogen cycling transformations within wetlands.

ACKNOWLEDGEMENTS

This chapter was completed in collaboration with Sarah Ludmer and Dr. Angela Kent. We would like to thank B. Stikkers and R. Weitekamp at the Champaign County Soil and Water District for logistical support in the field and maintenance of the St. Joseph Wetland. We extend our thanks to B. Wills, L. Endriukaitis for help in the field and M. Rout, D. Keymer, D. Li., Y. Lou, D. Lin, M. Porter, C. Smith, C. Mitchell, M. David for laboratory assistance. Y. Cao, J. Dalling, M. Wander, G. Spyreas, and S. Paver provided helpful comments to earlier versions of this manuscript. This work was supported by the Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under project number ILLU 875-374. This research was also supported, in part, by the Program in Ecology, Evolution, and Conservation Biology at the University of Illinois at Urbana-Champaign.

REFERENCES

Balser, T. C. and D. L. Wixon. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology **15**:2935-2949.

Bossio, D. A. and K. M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: Phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology **35**:265-278.

Cardinale, B. J., K. Nelson, and M. A. Palmer. 2000. Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos **91**:175-183.

Castenson, K. L. and M. C. Rabenhorst. 2006. Indicator of reduction in soil (IRIS): Evaluation of a new approach for assessing reduced conditions in soil. Soil Science Society of America Journal **70**:1222-1226.

Cavigelli, M. A. and G. P. Robertson. 2000. The functional significance of denitrifier community composition in a terrestrial ecosystem. Ecology **81**:1402-1414.

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology **12**:3137-3149.

Fierer, N. and J. P. Schimel. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal **67**:798-805.

Francis, C. A., J. M. Beman, and M. M. M. Kuypers. 2007. New processes and players in the nitrogen cycle: The microbial ecology of anaerobic and archaeal ammonia oxidation. ISME Journal **1**:19-27.

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proceedings of the National Academy of Sciences of the United States of America **102**:14683-14688.

Fraterrigo, J. M., T. C. Balser, and M. G. Turner. 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology **87**:570-579.

Hooper, D. U. and P. M. Vitousek. 1997. The effects of plant composition and diversity on ecosystem processes. Science **277**:1302-1305.

Jayakumar, A., G. D. O'Mullan, S. W. A. Naqvi, and B. B. Ward. 2009. Denitrifying bacterial community composition changes associated with stages of denitrification in oxygen minimum zones. Microbial Ecology **58**:350-362.

Jenkinson, B. J. and D. P. Franzmeier. 2006. Development and evaluation of iron-coated tubes that indicate reduction in soils. Soil Science Society of America Journal **70**:183-191.

Jessup, C. M., R. Kassen, S. E. Forde, B. Kerr, A. Buckling, P. B. Rainey, and B. J. M. Bohannan. 2004. Big questions, small worlds: microbial model systems in ecology. Trends In Ecology & Evolution **19**:189-197.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Kent, A. D., A. C. Yannarell, J. A. Rusak, E. W. Triplett, and K. D. McMahon. 2007. Synchrony in aquatic microbial community dynamics. ISME Journal **1**:38-47.

Kloos, K., A. Mergel, C. Rosch, and H. Bothe. 2001. Denitrification within the genus *Azospirillum* and other associative bacteria. Australian Journal of Plant Physiology **28**:991-998.

Kowalchuk, G. A. and J. R. Stephen. 2001. Ammonia-oxidizing bacteria: A model for molecular microbial ecology. Annual Review of Microbiology **55**:485-529.

Legendre, P. and L. Legendre. 1998. Numerical Ecology. Elsevier Science, Amsterdam.

Madigan, M. T. and J. M. Martinko. 2006. Brock Biology of Microorganisms. 11th edition. Pearson Prentice Hall, Upper Saddle River, New Jersey.

McArdle, B. H. and M. J. Anderson. 2001. Fitting multivariate models to community data: A comment on distance-based redundancy analysis. Ecology **82**:290-297.

Mentzer, J. L., R. M. Goodman, and T. C. Balser. 2006. Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie. Plant and Soil **284**:85-100.

Naeem, S., M. Loreau, and P. Inchausti. 2002. Biodiversity and ecosystem functioning: the emergence of a synthetic ecological framework. Pages pp. 3-11 *in* M. Loreau, S. Naeem, and P. Inchausti, editors. Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxform University Press, Oxford.

Naeem, S. and J. P. Wright. 2003. Disentangling biodiversity effects on ecosystem functioning: Deriving solutions to a seemingly insurmountable problem. Ecology Letters **6**:567-579.

Nicol, G. W., S. Leininger, C. Schleper, and J. I. Prosser. 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environmental Microbiology **10**:2966-2978.

Peralta, A. L., J. W. Matthews, and A. D. Kent. 2010. Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology **76**:4207-4215.

Pett-Ridge, J. and M. K. Firestone. 2005. Redox fluctuation structures microbial communities in a wet tropical soil. Applied and Environmental Microbiology **71**:6998-7007.

Prosser, J. I. 2007. The Ecology of Nitrifying Bacteria.*in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle. Elsevier, Oxford.

Reed, H. E. and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology **62**:161-170.

Rees, G. N., D. S. Baldwin, G. O. Watson, S. Perryman, and D. L. Nielsen. 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology **86**:339-347.

Rich, J. J., R. S. Heichen, P. J. Bottomley, K. Cromack, and D. D. Myrold. 2003. Community composition and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied and Environmental Microbiology **69**:5974-5982.

Rotthauwe, J. H., K. P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations. Applied and Environmental Microbiology **63**:4704-4712.

Royer, T. V., J. L. Tank, and M. B. David. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. Journal of Environmental Quality **33**:1296-1304.

Schimel, J., T. C. Balser, and M. Wallenstein. 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology **88**:1386-1394.

Sirivedhin, T. and K. A. Gray. 2006. Factors affecting denitrification rates in experimental wetlands: Field and laboratory studies. Ecological Engineering **26**:167-181.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Throback, I. N., K. Enwall, A. Jarvis, and S. Hallin. 2004. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiology Ecology **49**:401-417.

Tilman, D., J. Knops, D. Wedin, P. Reich, M. Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. Science **277**:1300-1302.

Van Cleemput, O., P. Boeckx, P. Lindgren, and K. Tonderski. 2007. Denitrification in Wetlands.*in* H. Bothe, S. J. Ferguson, and W. E. Newton, editors. Biology of the Nitrogen Cycle, Oxford.

Wallenstein, M. D., D. D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications **16**:2143-2152.

Wertz, S., V. Degrange, J. I. Prosser, F. Poly, C. Commeaux, N. Guillaumaud, and X. Le Roux. 2007. Decline of soil microbial diversity does not influence the resistance and resilience of key soil microbial functional groups following a model disturbance. Environmental Microbiology **9**:2211-2219.

Yannarell, A. C. and E. W. Triplett. 2005. Geographic and environmental sources of variation in lake bacterial community composition. Applied and Environmental Microbiology **71**:227-239.

Zak, D. R., C. B. Blackwood, and M. P. Waldrop. 2006. A molecular dawn for biogeochemistry. Trends In Ecology & Evolution **21**:288-295.

TABLES

Table 5.1. Summary of baseline soil factors and potential nitrification and denitrification rates (mean +/- standard deviation) according to hydrologic history (upland vs. wetland). Total organic carbon, total nitrogen, C:N ratio, and pH were analyzed from soils representing upland and wetland soils collected adjacent to soils in the present study.

variable	upland	wetland
total organic C	15.80 ± 2.07	29.75 ± 4.27
total nitrogen	1.20 ± 0.10	1.80 ± 0.51
C:N ratio	13.11 ± 0.06	17.21 ± 2.55
pH	7.56 ± 0.24	7.89 ± 0.08
moisture	17.48 ± 4.13	39.92 ± 5.22
nitrate-N	2.245 ± 0.167	12.053 ± 2.857
ammonium-N	0.643 ± 0.094	0.948 ± 0.193
Nitrification	5.192 ± 0.241	8.196 ± 0.368
Denitrification	170 ± 6	359 ± 25

Units for each variable: nitrification: moisture: %; nitrate: µg N g^{-1} soil; ammonium: µg N g^{-1} soil: ng NO_2^- g^{-1} dm hr^{-1}; denitrification: ng N_2O g^{-1} dm hr^{-1}

Tables 5.2. Summary of soil factors (mean +/- standard deviation) according to drying-flooding treatment. Values represent the change from original baseline value to post-treatment values for upland (a) and wetland (b) hydrologic history. Negative numbers indicate a decrease from baseline values and positive numbers indicate increase from baseline values.

(a) Upland hydrologic history

variable	dry	wet-dry	wet
moisture	-8.64 ± 1.04	11.38 ± 1.77	22.84 ± 1.25
nitrate-N	2.671 ± 1.419	2.133 ± 1.038	0.281 ± 0.073
ammonium-N	0.150 ± 0.154	0.003 ± 0.158	7.008 ± 0.365

(b) Wetland hydrologic history

variable	dry	wet-dry	wet
moisture	-25.51 ± 1.29	7.65 ± 2.29	9.29 ± 2.59
nitrate-N	2.591 ± 1.747	-6.859 ± 2.359	-8.553 ± 0.547
ammonium-N	-0.111 ± 0.171	0.287 ± 0.204	6.332 ± 1.587

Units for each variable: moisture: %; nitrate: μg N g^{-1} soil; ammonium: μg N g^{-1} soil

Table 5.3. Summary of analysis of variance (ANOVA) results. ANOVA of the main effects (hydrologic and drying/flooding treatment) and the interactions of main effects was carried out on differences in potential denitrification and nitrification rates (change in rate from pre- to post-drying/flooding treatment). Effects were considered significantly different at $P < 0.05$. *Post-hoc* contrasts are found in Table D.1.

(a) denitrification

effect	df	*F*-value	*P*-value
history	1, 18	15.29	0.0010
treatment	2, 18	96.46	<0.0001
history*treatment	2, 18	20.78	<0.0001

(b) nitrification

effect	df	*F*-value	*P*-value
history	1, 18	4.05	0.0595
treatment	2, 18	4.59	0.0245
history*treatment	2, 18	12.30	0.0004

Table 5.4. Summary of permutational multivariate analysis of variance (PERMANOVA) results. Contribution of hydrologic history and mesocosm drying-flooding treatments and the interaction on denitrifier (a) and ammonia oxidizer (b) community variation are reported. Effects were considered significantly influencing community composition at $P < 0.05$.

(a) denitrifier

effect	df	sum of sqs	F.model	R^2	*P*- value
history	1	4.5428	119.6420	0.6722	0.0010
treatment	3	0.2697	2.3680	0.0399	0.0390
history*treatment	3	0.1989	1.7460	0.0294	0.1089
residuals	46	1.7466	0.2585		
total	53	6.7581			

(b) ammonia oxidizer

effect	df	sum of sqs	F.model	R^2	*P*- value
history	1	0.1933	31.9710	0.3816	0.0010
treatment	3	0.0160	0.8800	0.0315	0.5385
history*treatment	3	0.0192	1.0580	0.0379	0.3706
residuals	46	0.2781	0.5490		
total	53	0.5066			

Table 5.5. Summary of analysis of variance (ANOVA) results. ANOVA of the main effects (hydrologic and drying/flooding treatment) and the interactions of main effects was carried out on differences in *nosZ* (a), archaeal *amoA* (AOA) (b), and bacterial *amoA* (AOB) (c) gene abundance (change in gene abundance from pre- to post-drying/flooding treatment). Effects were considered significantly different at $P < 0.05$.

(a) *nosZ* gene abundance

effect	df	*F*-value	*P*-value
history	1, 18	21.47	0.0002
treatment	2, 18	0.47	0.6328
history*treatment	2, 18	0.62	0.5503

(b) AOA gene abundance

effect	df	*F*-value	*P*-value
history	1, 18	78.72	<0.0001
treatment	2, 18	0.09	0.9125
history*treatment	2, 18	1.38	0.2769

(c) AOB gene abundance

effect	df	*F*-value	*P*-value
history	1, 18	10.41	0.0047
treatment	2, 18	1.19	0.3278
history*treatment	2, 18	1.47	0.2563

FIGURES

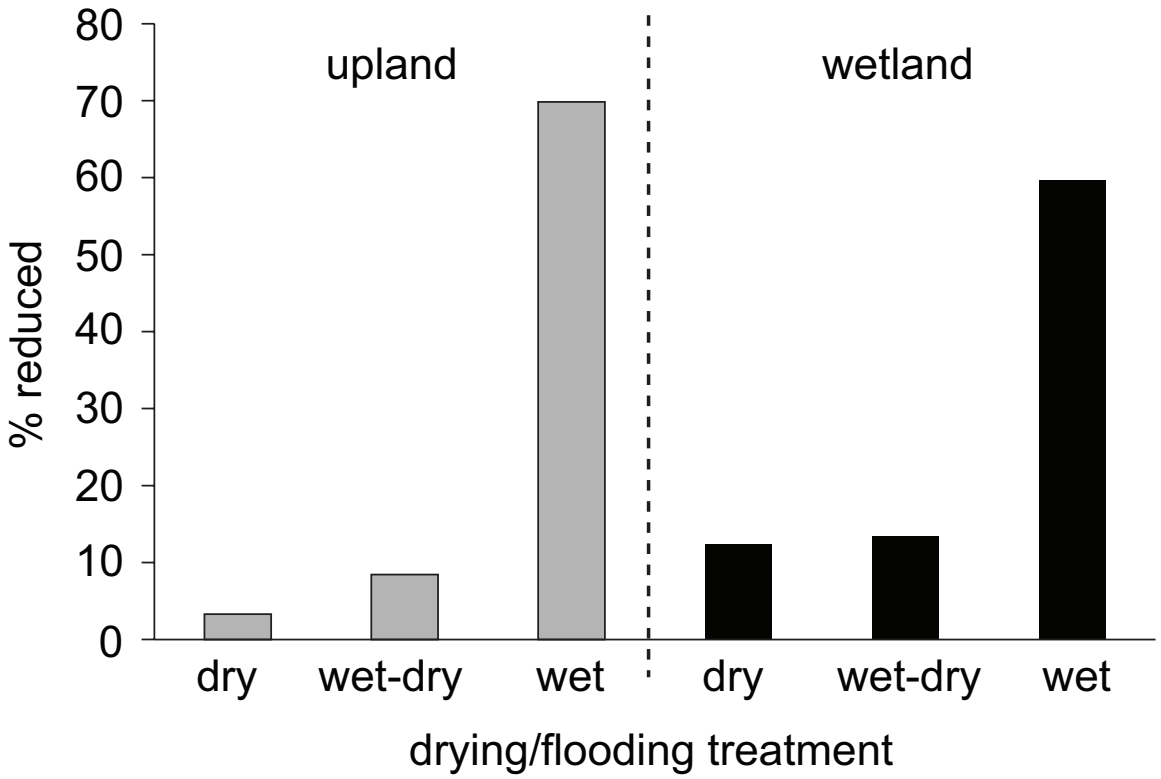


Figure 5.1. Soil redox status based on Indicator of Reduction in Soils (IRIS) tubes measured over the two-week drying-flooding treatment. Soil redox status was measured in pots according to hydrologic history (upland, wetland) and drying-flooding treatment (dry, wet-dry, wet). Reducing conditions are expressed as the percent of ferrihydrite paint reduced per IRIS tube. Dashed line represents separation by hydrologic history (upland vs. wetland).

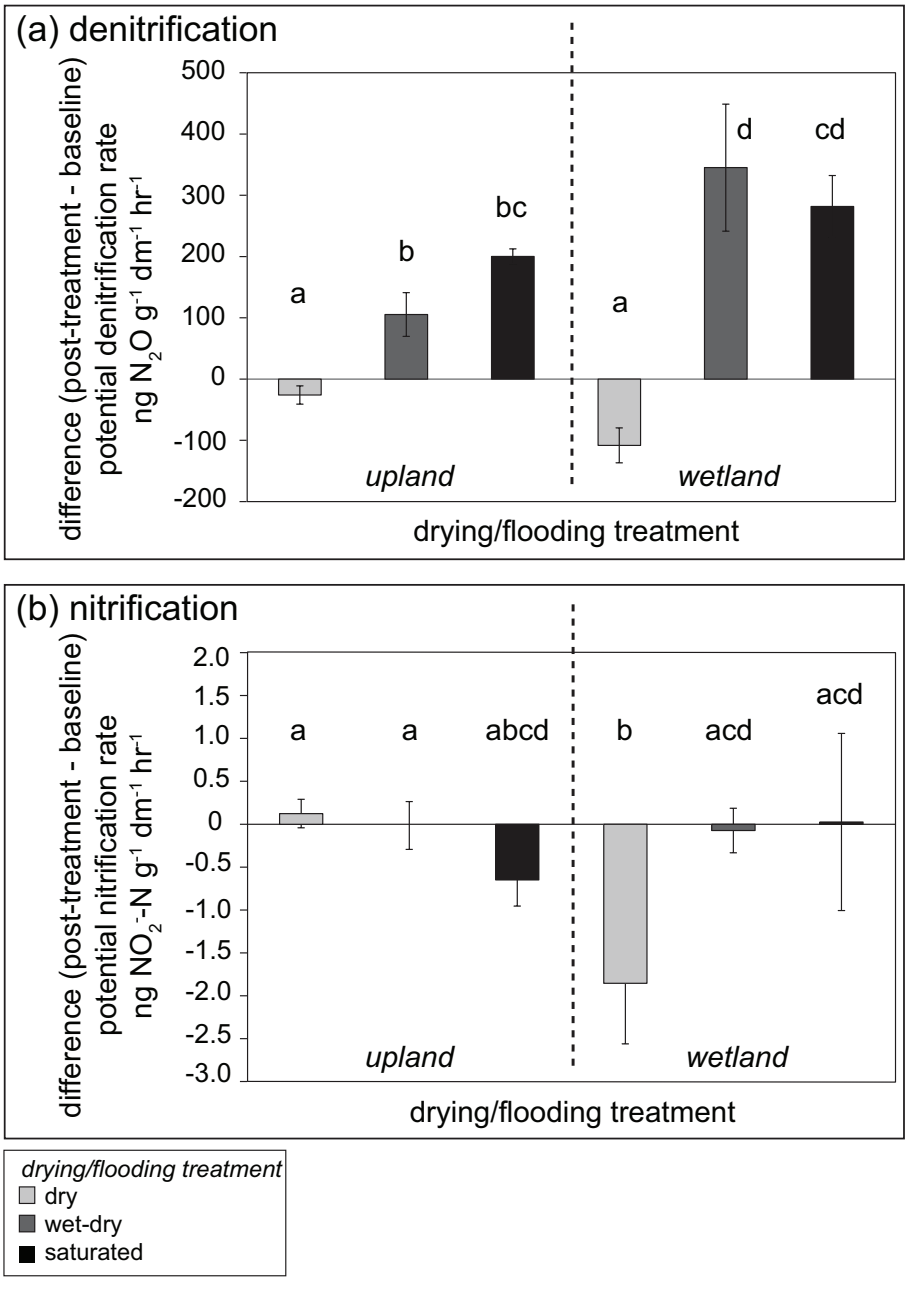


Figure 5.2. Mean (+/- standard deviation) of potential denitrification (a) and nitrification (b) rates from baseline to post-drying/flooding treatment (n = 4). Bars are colored by drying/flooding treatment (light gray = dry, dark gray = wet-dry, black = wet) and are grouped according to hydrologic history (upland vs. wetland). Different letters above bars are considered significantly different at $P < 0.05$. Dashed line represents separation by hydrologic history (upland vs. wetland).

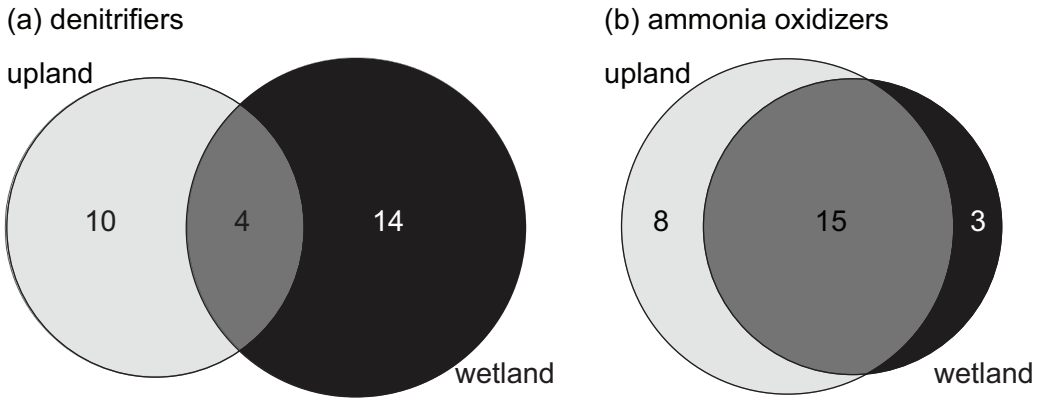


Figure 5.3. Two-way Venn diagrams based on denitrifier (a) and ammonia oxidizer (b) taxa visualizing taxa distribution and overlap between upland and wetland baseline soils based on presence/absence of T-RFs. The relationships within and among upland and wetland soils were scaled relative to the total number of taxa observed for each functional group (denitrifier: 28, ammonia oxidizer: 26). A taxon was considered present if detected in two out of four replicate pots within each drying-flooding treatment. About half of the T-RF's did not meet this criterion.

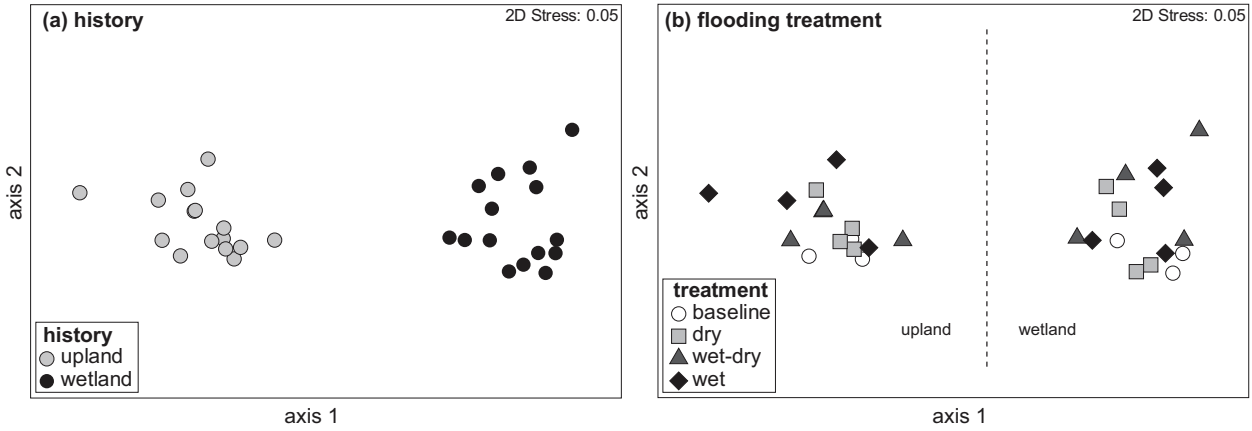


Figure 5.4. Non-metric multidimensional scaling (NMDS) plots of denitrifier community composition based on T-RFLP relative fluorescence. NMDS plots were labeled according to hydrologic history of soil (upland, wetland) (a) and according to drying/flooding treatment (baseline pre-treatment, dry, wet-dry, wet) (b). Baseline soils were composed of 3 replicates, and drying-flooding treatments were composed of 4 replicates. Symbols are colored gray and black circles represent communities from upland or wetland locations in (a). Symbols are colored white to black and differ in shape represent samples according to baseline and drying-flooding treatment (b). Dashed line represents separation by hydrologic history (upland vs. wetland).

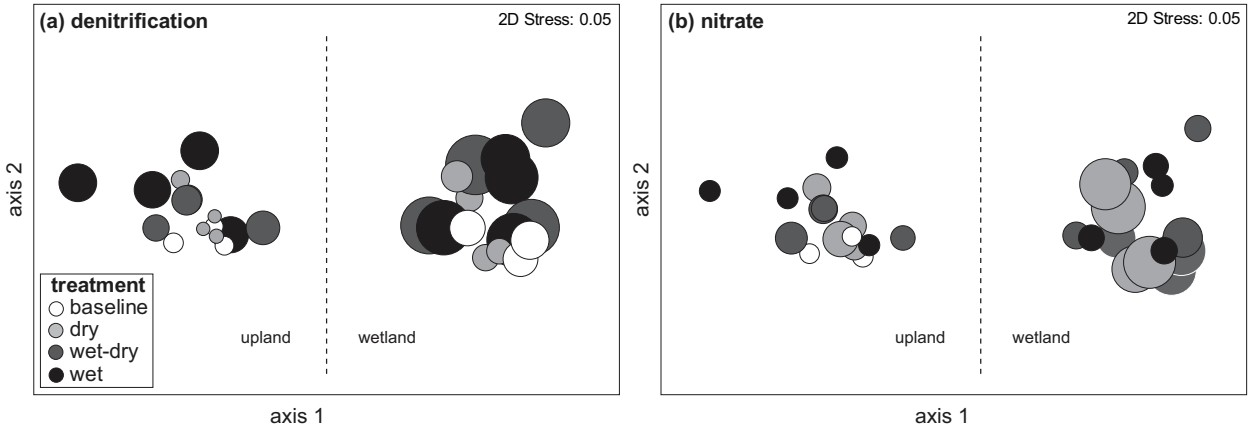


Figure 5.5. Bubble plots superimposed onto NMDS plots of denitrifier community composition. Bubble size corresponds to relative potential denitrification rate (a) and nitrate-N concentration (b) according to drying-flooding treatment (baseline, dry, wet-dry, wet) on 4 replicate pots (n = 4). Symbols are colored white to black represent samples according to baseline and drying-flooding treatment. Dashed line represents separation by hydrologic history (upland vs. wetland).

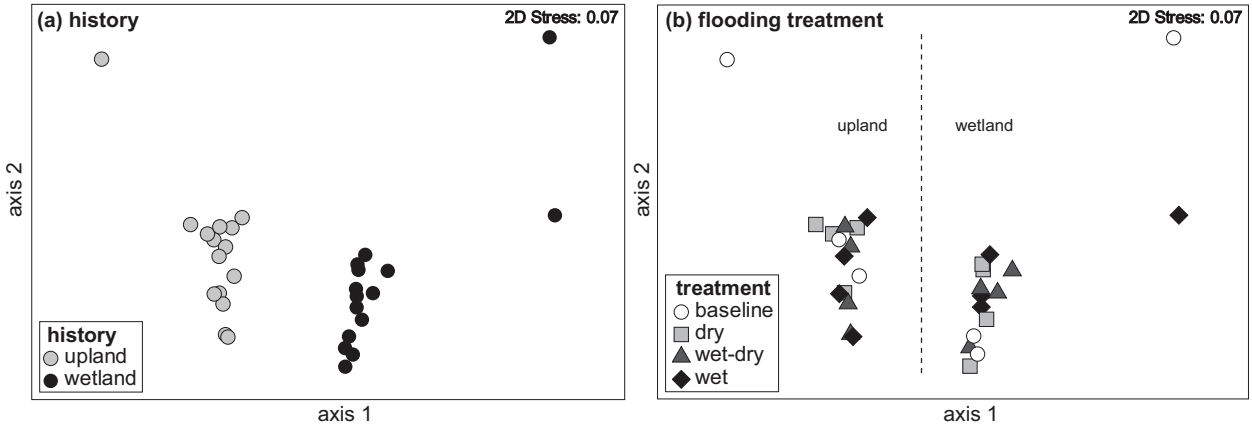


Figure 5.6. Non-metric multidimensional scaling (NMDS) plots of ammonia oxidizer community composition based on T-RFLP relative fluorescence. NMDS plots were labeled according to hydrologic history of soil (upland, wetland) (a) and according to drying/flooding treatment (baseline pre-treatment, dry, wet-dry, wet) (b). Baseline soils were composed of 3 replicates, and drying-flooding treatments were composed of 4 replicates. Symbols are colored gray and black circles represent communities from upland or wetland locations in (a). Symbols are colored white to black and differ in shape represent samples according to baseline and drying-flooding treatment (in B). Dashed line represents separation by hydrologic history (upland vs. wetland).

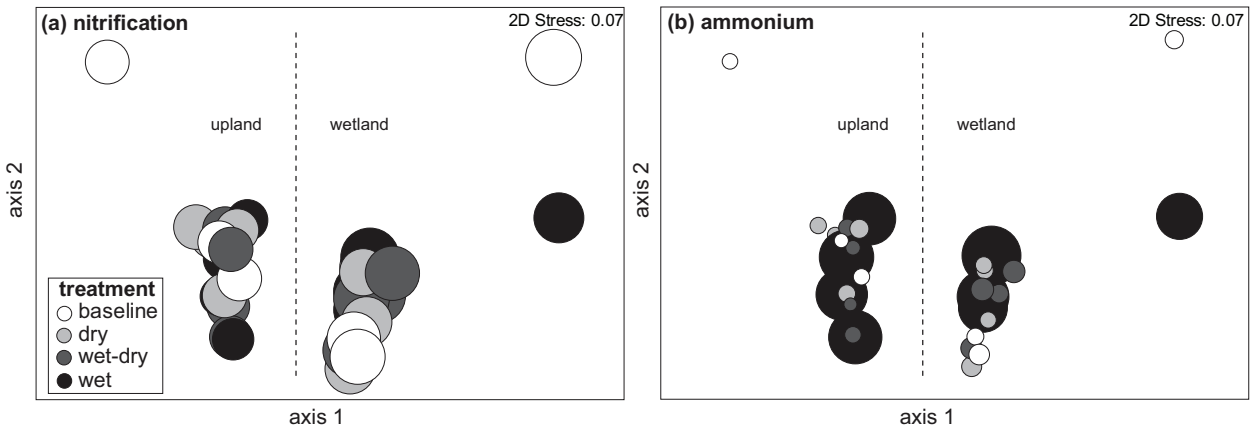


Figure 5.7. Bubble plots superimposed onto NMDS plots of ammonia oxidizer community composition. Bubble size corresponds to relative potential denitrification rate (a) and ammonium-N concentration (b) according to drying-flooding treatment (baseline, dry, wet-dry, wet) on 4 replicate pots (n = 4). Symbols are colored white to black represent samples according to baseline and drying-flooding treatment. Dashed line represents separation by hydrologic history (upland vs. wetland).

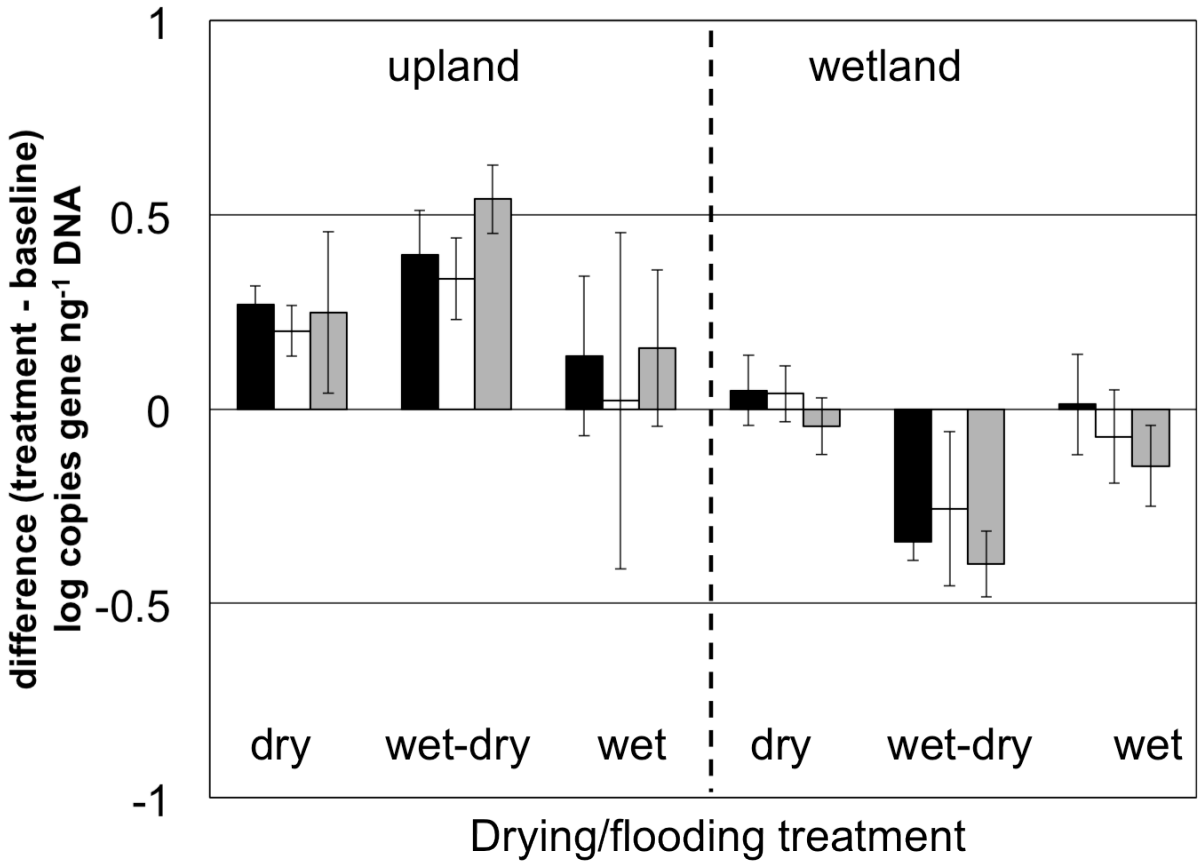


Figure 5.8. Mean (+/- standard deviation) of gene abundance from baseline to post-drying/flooding treatment for *nosZ* (black), bacterial *amoA* (white), and archaeal *amoA* (gray) genes (n = 4). Bars are grouped according to hydrologic history labeled at the top of the graph and separated by the dashed line (upland vs. wetland).

CHAPTER 6

CONCLUSION

The combination of wetland losses and increased agricultural use of synthetic fertilizers has exacerbated nutrient runoff. This nutrient runoff contributes to eutrophication in downstream waters. As a consequence, algal blooms are widespread, and are capable of supporting increased microbial decomposition, ultimately resulting in hypoxic zones, as seen most dramatically in coastal waters (Panno et al. 2008, Rabalais et al. 2002, Zedler 2003). Mitigation of nutrient loads can be accomplished by reducing synthetic fertilizer use, increasing wetland acreage, and enhancing nutrient cycling functions in restored wetlands (Zedler 2003).

Riparian wetlands support a range of biogeochemical transformations and play an important role in recycling nutrients directly impacting water quality (Keddy 2000). Microorganisms control nitrogen transformations that contribute to nitrate removal from surface waters and nitrate retention in wetland ecosystems; however, the link between microbial community structure and function in a restoration context is not clearly understood (Gutknecht et al. 2006). Varied responses by microorganisms can result in unexpected ecosystem-level consequences. Land use changes from agricultural to restored wetlands can greatly impact key ecosystem services, providing environmental conditions that support desired water quality functions such as denitrification. However, this functioning may be constrained by changes in hydrology and soil fertility from land use histories resulting in shifts in microbial community structure (Fraterrigo et al. 2006, Strickland et al. 2009). Uncertainty associated with the environment and microbial structure-function relationship has led to unpredictable and

potentially dramatic outcomes when restoring nutrient cycling functions (Reed and Martiny 2007, Wallenstein et al. 2006).

Microorganisms within communities are often assumed to be functionally redundant because of high species diversity and rapid response of microbes to their local environment (Goddard and Bradford 2003). However, evidence from recent studies has challenged the 'functional equivalence' hypothesis. Contrasting results suggest that distinct microbial community structure is accompanied by different microbial process rates when exposed to the same environmental conditions (i.e., substrate addition, temperature change) (Balser and Wixon 2009, DeAngelis et al. 2010, Reed and Martiny 2007, Strickland et al. 2009). The counter hypothesis of 'functional dissimilarity' predicts microbial community composition and environmental conditions, together, ultimately control ecosystem process rates (Balser and Firestone 2005, Strickland et al. 2009). In previous studies, ecosystem processes (e.g., carbon mineralization) carried out by broad groups of microbes were identified as more likely to exhibit 'functional equivalence' compared to more taxonomically specialized functions related to nitrogen transformations (Balser and Firestone 2005, Wertz et al. 2006). In this dissertation, I tested the 'functional dissimilarity' hypothesis by comparing microbial composition and activity between two functional groups involved in wetland nitrogen cycling. I used observational and experimental approaches over different spatial scales to investigate the relationships among environmental factors, community structure, and ecosystem function. Differences in microbial responses can complicate restoration outcomes in circumstances where a desired ecosystem services (e.g., denitrification) is supported by the same environmental conditions as ecosystem disservices (e.g., methane production, nitrification).

Microbial systems are ideal for studying the structure-function relationships. Molecular microbial analyses target genes encoding enzymes necessary for nutrient cycling transformations. Complementary potential enzyme assays analyze activity of the resident microbial assemblage under controlled, non-limiting nutrient conditions in the laboratory. In wetland ecosystems, denitrification and nitrification are important nitrogen transformations carried out by different microbial functional guilds. Molecular microbial ecology methods allow us to characterize microbial communities involved in denitrification and nitrification through targeting the nitrous oxide reductase (*nosZ*) gene and the ammonia monooxygenase (*amoA*) gene, respectively. The goal of this study was to determine potential predictors of nitrate removal function in restored wetlands. Evidence from my work suggests that microorganisms vary in their sensitivity to soil factors, resulting in differences in microbial activity potential constrained by the resident microbial community. Variation in microbial response to local soil conditions can result in shifts in nitrogen cycling transformations within wetlands.

CHAPTER SUMMARIES

In Chapter 2, an ecological filter concept was used to identify potential abiotic factors important for structuring wetland plant and microbial communities. The relationship among landscape and local soil factors, plant communities, total bacterial and denitrifier communities, was assessed. Different climate and regional variables were associated with plant and microbial communities. Plant community composition was significantly associated with land use surrounding wetlands and inorganic nitrogen levels, while local soil factors, specifically soil pH, was significantly linked to microbial community composition in restored wetlands. These results suggest that different environmental variables can significantly affect plant and microbial

communities, and identifying critical factors correlated with desired ecosystem functions can help guide restoration management or restoration site placement to support specific wetland functions (e.g., habitat provision, nutrient cycling). However, the wetland survey did not provide evidence that the abiotic factors significantly influenced ecosystem functions because microbial activity was not assessed. From the restored wetlands survey, I identified environmental factors, namely soil moisture, redox conditions and nitrogen availability that contributed significantly to variation in microbial community composition.

Based on the wetlands survey study, I then aimed to assess the influence of a characterized environmental gradient on nitrogen cycling microbial functional groups. Specifically, in Chapter 3, I examined the relationship between abiotic factors and composition of denitrifying and ammonia oxidizing microorganisms along a soil moisture gradient. I hypothesized that facultatively anaerobic denitrifiers and aerobic ammonia oxidizers would have different sensitivity to oxygen concentration/soil moisture levels. My results showed that both denitrifier and ammonia oxidizer community composition significantly differed along the environmental gradient, and denitrifier taxa were observed more frequently over a wider moisture range compared to ammonia oxidizer taxa. If microbial functional groups vary in environmental tolerance, the functions associated with these microbes may also be affected. Soil moisture was not the sole abiotic factor important to microbial community composition along the gradient as soil texture, pH, and total organic carbon were important factor corrected to microbial community composition.

To further examine microbial response to environmental gradients, I assessed the relationship between denitrifier and ammonia community composition and activity along a hydrologic gradient within wetlands characterized by different physical and chemical gradients.

Specifically, in Chapter 4, I further explored the links among local soil factors and microbial community structure and function within different wetlands to better understand how long-term changes in environmental conditions compared to short-term changes within a season influenced microbial community composition and function. I investigated denitrifier and ammonia oxidizer community composition and activity over a hydrologic gradient, where other soil factors also varied along the gradient within each wetland. Microbial composition was assumed to be a function of the local environment, and microbial activity was a function of the local environment and community composition. Potential denitrification and nitrification rates were mainly influenced by environmental conditions compared to community composition or denitrifier/ammonia oxidizer gene abundance (assessed by qPCR). When focusing on the influence of community composition alone, nitrification was influenced by community composition more than denitrification. Unexpected outcomes, namely reduced nitrification function under dry, aerobic conditions were accompanied by low soil pH (below pH 6.5), levels that have been documented to inhibit nitrification activity. In addition, reduced denitrification function in saturated conditions was associated with low soil fertility (nitrate, soil organic carbon). Based on results from natural environmental gradients in this study, it was unclear whether abiotic factors identified as important to microbial structure and function would remain important drivers over time.

From the observational surveys at the landscape and local levels, it was not possible to assess the direct influence of changing moisture moisture/redox conditions on denitrifier and ammonia oxidizer community composition and activity. In order to isolate the effect of soil moisture on microbial community structure and function related to N cycling, I experimentally manipulated upland and wetland soils from a single restoration site. Since community assembly

can be influenced by past and contemporary environmental conditions (Chase 2010, Reed and Martiny 2007), I experimentally assessed the role of hydrologic history as it pertained to denitrifiers and ammonia oxidizers responding to current drying/flooding conditions in Chapter 5. Recent studies have provided evidence that current environmental conditions can result in functionally different communities when these communities developed under different environmental histories (Balser and Firestone 2005, Reed and Martiny 2007, Strickland et al. 2009). Hydrology strongly influences microbial communities (Gutknecht et al. 2006), and historic environmental conditions can potentially constrain microbial functions. Soils sampled from different hydrologic histories (upland and wetland locations) within a single wetland were exposed to drying/flooding treatments (dry, wet-dry, wet). Soil fertility, pH, and moisture were higher in wetland compared to upland sites, and these baseline conditions supported higher potential denitrification and nitrification rates. Results from the drying/flooding treatments revealed that potential denitrification rates significantly increased under wetter soil conditions, while nitrification rates remained stable in many instances. This difference in microbial response could result in unwanted outcomes in N cycling; suggesting a significant potential link between restoration, soil communities, and ecosystem-level functioning. Overall, denitrifier and ammonia oxidizer community composition differenced according to hydrologic history, before and after the short-term drying/flooding treatment. A small but significant relationship between drying/flooding treatment and denitrifier community composition was observed, whereas no effect of treatment on ammonia oxidizer communities was observed. This study revealed that the differential response of microbial functional guilds to disturbances may contribute to shifts in nitrogen cycling transformations. If denitrification rates are limited during dry-down periods but nitrification rates remain constant, nitrate levels may build up and potentially leach out of the

wetland instead of getting processed into nitrogen gas. Variations in microbial functional response and constraints due to hydrologic history can result in unwanted shifts in nutrient cycling activities.

To expand on the wetlands survey study, a survey at a subset of sites characterized as high or low site fertility can be used to compare plant communities and associated functions (e.g., biomass production) and sites where soil pH ranges from acidic to the alkaline conditions can be used to compare microbial community composition and rates of nutrient cycling functions (i.e., carbon and nitrogen cycling processes). Assessment of plant and microbial community structure and function over time could test the strength of environmental filters on wetland communities. To further elucidate environmental tolerance of denitrifier and ammonia oxidizers in the environment, experimental manipulation of environmental conditions is necessary. For example, a common garden experiment where microorganisms were isolated and placed along a well-characterized gradient in the field or a controlled gradient in a laboratory setting could provide a way to test denitrifier and ammonia oxidizer environmental tolerance more directly. Common garden experiments alongside experimental treatment experiments can provide a clearer test of causative agents acting on microbial functional response (Reed and Martiny 2007). Limitations associated with the drying/flooding experiment included spatial scale. This drying/flooding experiment had limited inference space because soils were collected within a single restored wetland. Increasing inference space to better inform restoration management, ecosystem-level manipulations of hydrology in a background of varying hydrologic histories, would provide a scaled-up version of the experiment performed in this dissertation. Hydrologic history can be engineered by controlling the periodicity and intensity of flooding events with dams or weirs. Land use changes can be used as additional imposed treatment effects. However,

comparisons between laboratory and field data in my work were compelling and suggest a strong influence of hydrologic history and land use that warrants larger-scale investigation. Monitoring microbial response over time can allow for the study of short-term and long-term effects on structure and function. Meaning that we can then begin to understand if functional constraints observed in this study were short-term effects that would be followed by long-term changes given time. This has direct implications for monitoring restoration and achieving restoration goals related to water quality function.

Additional studies could also include examining *in situ* structure-function relationships through taking advantage of RNA-based molecular approaches along with stable isotope approaches (Dijkstra et al. 2008, Manefield et al. 2002, Neufeld et al. 2007, Paterson et al. 2009, Vandenkoornhuyse et al. 2007, Whiteley et al. 2007). The microbial community structure-function relationships may vary based on observations of the 'active' compared to 'potential' community. The analysis of the 'active' community is more likely to capture short-term microbial community changes in response to sudden environmental perturbations. Changes in community structure may be more obvious if the 'active' microbial community is targeted. The current method of T-RFLP was used to assess denitrifier and ammonia oxidizer community composition is unable to track short-term, subtle changes in community composition. This DNA-based molecular method does a good job of assessing general patterns in community composition over space and time (Schutte et al. 2008). In a previous study, comparing 16S bacterial RNA- and DNA-based T-RFLP approaches showed correlated patterns, where the RNA approach resulted in more distinct differences in an agricultural compared to serpentine soil treated with toxic levels of chromate (Mengoni et al. 2005). Another study comparing DNA-based and mRNA T-RFLP of the particulate methane monooxygenase (*pmoA*) gene to detect

methanotrophs in the environment showed a distinct community difference between the DNA and mRNA-based T-RFLP approach (Krause et al. 2010). Despite the total and the active communities both changing over time, the methane oxidation rates remained constant, providing evidence that different taxa maintain this ecosystem function (Krause et al. 2010). Including RNA-based information can provide us with a more realistic view of what is going on in the short-term and used to predict what will occur under future environmental conditions.

FINAL REMARKS

In this dissertation, an observational approach at different spatial scales was used to generate hypotheses at a local wetland. Microbial community structure and function were examined using environmental treatment approach along natural environmental gradients, and experimental manipulations of drying/flooding conditions were used to examine relationships between environmental influences on microbial structure-function relationships by comparing two microbial functional groups. In total, my work suggests that prior land use history and current environmental conditions impact microbial communities differentially, and constraints on microbial functions are due to resident microbial community structure and contemporary environmental conditions. Results suggest that denitrifier and ammonia oxidizer communities vary in response to environmental conditions and influence associated nitrogen cycling activities. Denitrification was more sensitive to contemporary changes in environmental conditions than nitrification. Microbial functions are influenced by community composition and the local environment to different degrees, making it challenging to predictive microbial response to changes in land use and environmental conditions

REFERENCES

Balser, T. C. and M. K. Firestone. 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry **73**:395-415.

Balser, T. C. and D. L. Wixon. 2009. Investigating biological control over soil carbon temperature sensitivity. Global Change Biology **15**:2935-2949.

Chase, J. M. 2010. Stochastic Community Assembly Causes Higher Biodiversity in More Productive Environments. Science **328**:1388-1391.

DeAngelis, K. M., W. L. Silver, A. W. Thompson, and M. K. Firestone. 2010. Microbial communities acclimate to recurring changes in soil redox potential status. Environmental Microbiology **12**:3137-3149.

Dijkstra, P., C. M. LaViolette, J. S. Coyle, R. R. Doucett, E. Schwartz, S. C. Hart, and B. A. Hungate. 2008. N-15 enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function. Ecology Letters **11**:389-397.

Fraterrigo, J. M., T. C. Balser, and M. G. Turner. 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology **87**:570-579.

Goddard, M. R. and M. A. Bradford. 2003. The adaptive response of a natural microbial population to carbon- and nitrogen-limitation. Ecology Letters **6**:594-598.

Gutknecht, J. L. M., R. M. Goodman, and T. C. Balser. 2006. Linking soil process and microbial ecology in freshwater wetland ecosystems. Plant and Soil **289**:17-34.

Keddy, P. A. 2000. Wetland Ecology: Principles and Conservation. Cambridge Studies in Ecology.

Krause, S., C. Luke, and P. Frenzel. 2010. Succession of methanotrophs in oxygen–methane counter-gradients of flooded rice paddies. ISME Journal **4**:1603-1607.

Manefield, M., A. S. Whiteley, R. I. Griffiths, and M. J. Bailey. 2002. RNA stable isotope probing, a novel means of linking microbial community function to Phylogeny. Applied and Environmental Microbiology **68**:5367-5373.

Mengoni, A., E. Tattie, F. Decorosi, C. Viti, M. Bazzicalupo, and L. Giovannetti. 2005. Comparison of 16S rRNA and 16S rDNA T-RFLP approaches to study bacterial communities in soil microcosms treated with chromate as perturbing agent. Microbial Ecology **50**:375-384.

Neufeld, J. D., M. Wagner, and J. C. Murrell. 2007. Who eats what, where and when? Isotope-labelling experiments are coming of age. ISME Journal **1**:103-110.

Panno, S. V., W. R. Kelly, K. C. Hackley, H. H. Hwang, and A. T. Martinsek. 2008. Sources and fate of nitrate in the Illinois River Basin, Illinois. Journal of Hydrology **359**:174-188.

Paterson, E., A. J. Midwood, and P. Millard. 2009. Through the eye of the needle: a review of isotope approaches to quantify microbial processes mediating soil carbon balance. New Phytologist **184**:19-33.

Rabalais, N. N., R. E. Turner, and D. Scavia. 2002. Beyond science into policy: Gulf of Mexico hypoxia and the Mississippi River. Bioscience **52**:129-142.

Reed, H. E. and J. B. H. Martiny. 2007. Testing the functional significance of microbial composition in natural communities. FEMS Microbiology Ecology **62**:161-170.

Schutte, U. M. E., Z. Abdo, S. J. Bent, C. Shyu, C. J. Williams, J. D. Pierson, and L. J. Forney. 2008. Advances in the use of terminal restriction fragment length polymorphism (T-

RFLP) analysis of 16S rRNA genes to characterize microbial communities. Applied Microbiology And Biotechnology **80**:365-380.

Strickland, M. S., C. Lauber, N. Fierer, and M. A. Bradford. 2009. Testing the functional significance of microbial community composition. Ecology **90**:441-451.

Vandenkoornhuyse, P., S. Mahe, P. Ineson, P. Staddon, N. Ostle, J. B. Cliquet, A. J. Francez, A. H. Fitter, and J. P. W. Young. 2007. Active root-inhabiting microbes identified by rapid incorporation of plant-derived carbon into RNA. Proceedings of the National Academy of Sciences of the United States of America **104**:16970-16975.

Wallenstein, M. D., D. D. Myrold, M. Firestone, and M. Voytek. 2006. Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. Ecological Applications **16**:2143-2152.

Wertz, S., V. Degrange, J. I. Prosser, F. Poly, C. Commeaux, T. Freitag, N. Guillaumaud, and X. Le Roux. 2006. Maintenance of soil functioning following erosion of microbial diversity. Environmental Microbiology **8**:2162-2169.

Whiteley, A. S., B. Thomson, T. Lueders, and M. Manefield. 2007. RNA stable-isotope probing. Nature Protocols **2**:838-844.

Zedler, J. B. 2003. Wetlands at your service: reducing impacts of agriculture at the watershed scale. Frontiers In Ecology And The Environment **1**:65-72.

APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER 2

Table A.1. Site description for restored wetlands sampled in June 2006 (from Matthews et al. 2008 appendix). Region refers to the location of each wetland in Illinois. Detailed site description found in Matthews et al. (2008).

County	Age in 2006	Latitude	Longitude	Region	Area (ha)	Perimeter (m)	Plant cover
Cook	14	41.57	-87.89	North	0.90	606.93	Herbaceous
Cook	14	42.01	-88.20	North	0.79	730.49	Herbaceous
Henderson	9	40.82	-91.07	North	3.55	989.9	Herbaceous
Henry	9	41.55	-90.19	North	6.53	1264.55	Herbaceous
JoDaviess	8	42.42	-90.40	North	3.13	888.16	Herbaceous
Lake	12	42.17	-87.97	North	0.87	432.64	Herbaceous
Mercer	9	41.25	-90.59	North	0.66	317.93	Forest
Ogle	11	41.89	-89.41	North	2.08	1095.38	Herbaceous
Ogle	11	41.89	-89.42	North	1.40	500.82	Forest
Stephenson	6	42.46	-89.65	North	3.89	642.22	Herbaceous
Stephenson	6	42.46	-89.65	North	3.86	1497.5	Forest
Whiteside	11	41.84	-89.70	North	1.04	493.61	Herbaceous
Cass	9	40.07	-90.30	Central	1.78	1270.54	Forest
Champaign	10	39.95	-88.27	Central	1.33	3346.78	Herbaceous
Hancock	14	40.41	-90.95	Central	1.34	545.82	Forest
Macon	5	39.76	-88.98	Central	4.01	1085.95	Herbaceous
Pike	9	39.73	-91.35	Central	7.07	1483.72	Herbaceous
Sangamon	9	39.84	-89.65	Central	1.61	1572.32	Herbaceous
Sangamon	6	39.88	-89.67	Central	2.40	1578.49	Herbaceous
Alexander	9	37.29	-89.51	South	1.01	515.57	Forest
Clinton	8	38.52	-89.63	South	2.72	677.39	Forest
Jackson	4	37.70	-89.22	South	0.53	467.46	Herbaceous
Jackson	6	37.79	-89.23	South	2.53	589.52	Herbaceous
Madison	5	38.82	-89.98	South	3.61	773.12	Herbaceous
Perry	5	38.01	-89.37	South	1.58	841.21	Forest
Saline	9	37.74	-88.70	South	1.56	529.86	Forest
St. Clair	12	38.57	-90.13	South	0.18	180.55	Herbaceous

Table A.2. Minimum, maximum and range of landscape and local soil factors across 27 restored wetlands.

variable	minimum	maximum	range
GDD	2511	4513	2002
precipitation	884	1182	298
temperature	7.39	14.00	6.61
moisture	16.07	61.74	45.67
rate Fe	0.06	3.68	3.61
C/N	8.49	34.73	26.24
TOC	5.78	47.95	42.17
TN	0.67	3.07	2.40
pH	5.38	8.03	2.65
available NO_3^--N	0.34	1.47	1.13
available NH_4^+-N	0.39	1.72	1.33

ABBREVIATIONS – GDD: growing degree days; rate Fe: redox status based on IRIS tube analysis; C/N: soil carbon to nitrogen ratio (wt/wt); TOC: total organic carbon; TN: total nitrogen
UNITS – precipitation: mm; temperature: °C; TOC: g C kg^{-1} soil; TN: g N kg^{-1} soil; CN: wt/wt; moisture: %; ammonium: μg NH_4^+-N g^{-1} soil; nitrate: μg NO_3^--N g^{-1} soil

APPENDIX B

SUPPLEMENTARY INFORMATION FOR CHAPTER 3

Table B.1. Summary of percent contribution of plant species to Bray-Curtis similarities among samples within a position along the moisture gradient using the similarity percentages routine (SIMPER). Only plant species that contribute over 5% to the similarity within each group are included.

Plot A Average similarity: 43.62

Species	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum%
Conyza canadensis	0.62	37.31	1.12	85.52	85.52
Elymus virginicus	0.09	2.73	1.76	6.25	91.77

Plot B Average similarity: 41.86

Species	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum%
Ambrosia artemisiifolia	0.35	25.21	2.59	60.22	60.22
Conyza canadensis	0.05	2.75	1.26	6.56	66.79
Elymus virginicus	0.05	2.65	1.63	6.33	73.12
Erigeron annuus	0.08	2.31	1.06	5.52	78.63

Plot C Average similarity: 78.74

Species	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum%
Setaria faberi	0.66	58.66	6.68	74.50	74.50
Conyza canadensis	0.21	17.45	7.73	22.16	96.66

Table B.2. Summary of percent contribution of *nosZ* T-RFs to Bray-Curtis similarities among samples within a position along the moisture gradient using the similarity percentages routine (SIMPER). Only *nosZ* T-RFs that contribute over 5% to the similarity within each group are included.

Plot A Average similarity: 46.87

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
H-225	0.36	13.34	3.56	28.46	28.46
A-413	0.29	11.58	28.26	24.70	53.15
H-112	0.10	3.11	1.10	6.63	59.78
A-203	0.11	2.97	0.99	6.33	66.11
A-288	0.16	2.88	0.57	6.13	72.24

Plot B Average similarity: 54.55

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
H-225	0.26	10.94	6.14	20.05	20.05
A-413	0.17	7.37	8.09	13.51	33.57
H-213	0.10	3.69	1.76	6.77	40.33
A-289	0.12	2.99	0.91	5.49	45.82
H-112	0.10	2.93	1.58	5.38	51.20
H-246	0.09	2.81	9.39	5.15	56.35

Plot C Average similarity: 73.53

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
A-413	0.26	11.63	12.33	15.82	15.82
H-225	0.21	10.11	75.64	13.74	29.56
H-213	0.14	6.40	8.07	8.71	38.27
A-376	0.12	5.01	5.17	6.82	45.09
A-289	0.11	4.72	38.09	6.42	51.50
H-113	0.09	4.06	5.53	5.52	57.03

Plot D Average similarity: 76.94

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
A-413	0.51	23.48	7.71	30.51	30.51
H-225	0.27	11.51	6.94	14.96	45.47
A-416	0.1	4.06	9.7	5.27	50.74
A-203	0.1	4.06	3.12	5.27	56.01
H-315	0.1	3.97	3.91	5.16	61.17
H-224	0.1	3.87	2.94	5.03	66.2
H-190	0.09	3.84	7.78	4.99	71.19

Table B.3. Summary of percent contribution of *amoA* (S3) T-RFs to Bray-Curtis similarities among samples within a position (plot A-D) along the moisture gradient using the similarity percentages routine (SIMPER). Only *amoA* T-RFs that contribute over 5% to the similarity within each group are included.

Plot A Average similarity: 30.47

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
G-572	0.25	7.14	0.90	23.42	23.42
Y-194	0.40	5.63	0.44	18.48	41.90
Y-337	0.22	4.23	0.94	13.89	55.80
G-204	0.14	2.92	0.74	9.60	65.39
G-197	0.12	2.32	0.56	7.60	72.99
G-638	0.07	1.65	0.91	5.40	78.40

Plot B Average similarity: 66.53

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
G-572	0.51	20.47	3.47	30.77	30.77
Y-337	0.41	19.09	44.15	28.69	59.46
G-197	0.20	7.66	5.26	11.52	70.97
Y-194	0.21	4.14	0.66	6.22	77.19
Y-639	0.09	3.90	5.07	5.86	83.05

Plot C Average similarity: 68.32

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
Y-571	0.38	15.85	2.79	23.20	23.20
G-638	0.41	13.80	1.76	20.21	43.40
G-205	0.41	13.34	1.87	19.52	62.92
Y-639	0.21	6.90	1.64	10.10	73.02
Y-141	0.18	6.16	2.74	9.01	82.03

Plot D Average similarity: 89.52

T-RF	Avg Abund	Avg Simil	Simil/SD	Contrib%	Cum.%
Y-431	0.31	14.26	15.81	15.93	15.93
Y-337	0.28	13.53	18.01	15.11	31.04
Y-439	0.30	12.77	7.11	14.26	45.30
G-197	0.26	11.78	5.72	13.15	58.45
G-300	0.19	9.19	18.07	10.27	68.72
G-284	0.18	8.72	52.89	9.74	78.47
G-205	0.17	7.83	10.46	8.75	87.22
G-638	0.12	4.84	4.14	5.41	92.63

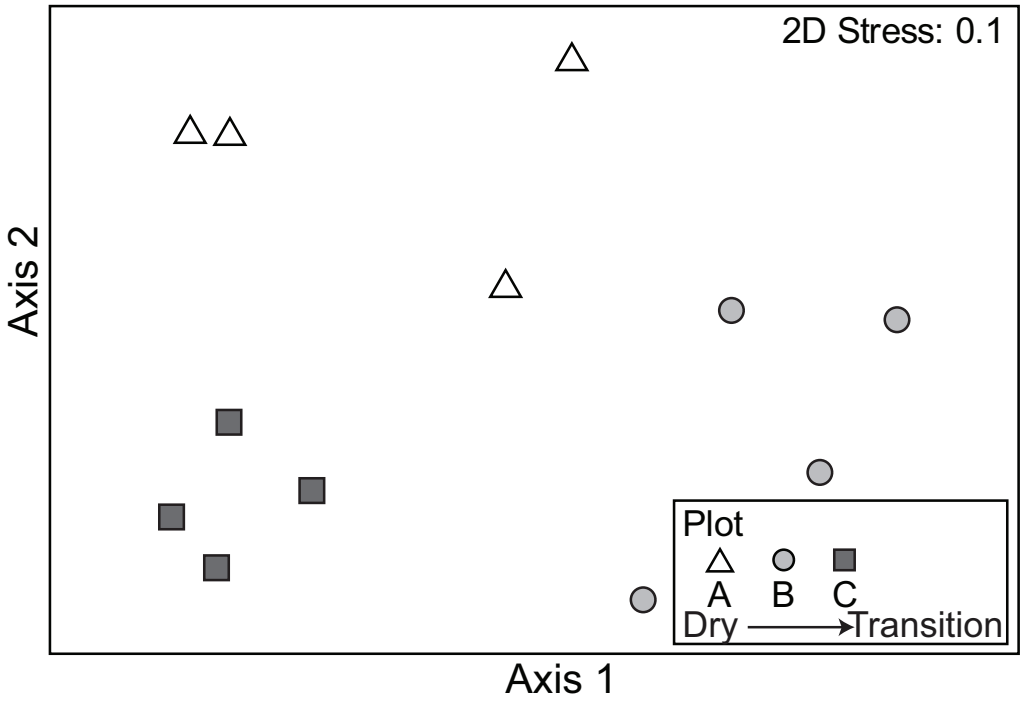


Figure B.1. Non-metric multidimensional scaling plot of plant community composition averaged over four months at plots along the moisture gradient at Emiquon Preserve. Symbols are colored white to dark gray and differ in shape to represent samples along an increasing moisture gradient. Each point represents community composition of plants based on relative abundance of species present within a 1-m^2 plot. No emergent vegetation was observed in the most saturated plots (Plot D).

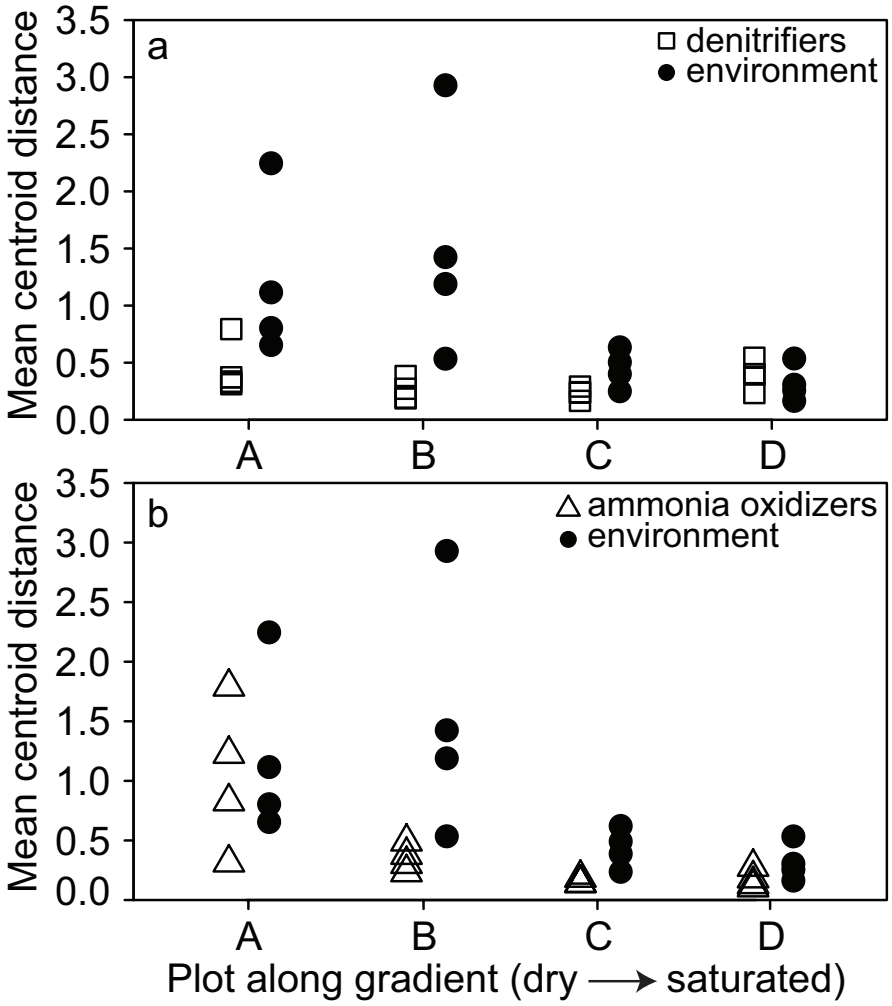


Figure B.2. Summary of dispersion based on mean centroid distances calculated from axis 1 and 2 from principle components analysis of the environmental factors (closed circles), axis 1 and 2 of correspondence analyses for denitrifiers (open squares) (a) and ammonia oxidizers (open triangles) (b). The higher the value, the more variable the individual points are from the centroid along the moisture gradient. Dispersion was averaged along the gradient over all months.

Statistical analyses

Summarizing environmental parameters along moisture gradient

Principle components analysis (PCA) was carried out on soil factors (% moisture, % total C, % total N, % organic matter, pH, % sand, % silt, and % clay. The historical soil moisture regime was treated as more important for structuring microbial communities than immediate changes in soil moisture. Therefore, soil moisture was averaged across the growing season and

included as an explanatory variable in the PCA. Each environmental variable was standardized using a z-score transformation prior to running PCA. This method provided an ordination of the samples based on environmental data.

Assessing microbial community variability along the environmental gradient

Correspondence analysis was performed on standardized microbial community data generated from *nosZ* and *amoA* T-RFLP for all collected soil samples (64) using Canoco 4.5.1 (Biometrics-Plant Research International, Wageningen, The Netherlands) {ter Braak, 2002 #47}. Month was treated as a covariable, and variation due to month was removed prior to running correspondence analysis, resulting in comparison over the gradient only, which was similar to the environmental analysis.

Environmental variability was based on the first two principal components and microbial community variability was based on the first two correspondence axes. Scores along the first two major axes were used to summarize the variability in environmental parameters and microbial communities along the gradient by calculating the spread or dispersion of each sample from the centroid of samples grouped by location along the transect (plots A-D). Spearman rank correlation was carried out using the average centroid distance calculated for the environmental parameters as well as the microbial communities (averaged over four months) for each location along the gradient (n = 16).

Results and Discussion: *Microbial community variability along a moisture gradient*

Environmental and microbial community heterogeneity/dispersion (based on spread of PCA or CA points, respectively) were calculated to represent variability in environmental parameters or community composition along the moisture gradient (Fig. S1). Environmental

heterogeneity (represented as the spread of PCA scores within a group of samples) decreased from dry to saturated plots (Fig. S1); that is, saturated plots among all transects were more similar to each other in environmental conditions than the dry, upland plots were to each other. Variability in denitrifier community composition (indicated by mean centroid distance within a group based on CA axis scores) did not correspond to the moisture gradient. No relationship was observed between environmental and denitrifier variability (Spearman's $\rho = -0.12$, $P = 0.6643$) (Fig. S1A). Similar to the environmental heterogeneity, ammonia oxidizer communities were also quite variable among the dry, upland plots. In contrast, ammonia oxidizer communities were similar among the saturated plots (Fig. 4B). A significant correlation was observed between environmental variability and ammonia oxidizer variability (Spearman's $\rho = 0.72$, $P = 0.0017$). Denitrifier and ammonia oxidizer community composition differed along the gradient, and these results suggest the distribution of ammonia oxidizers was influenced more by the local environment than denitrifiers.

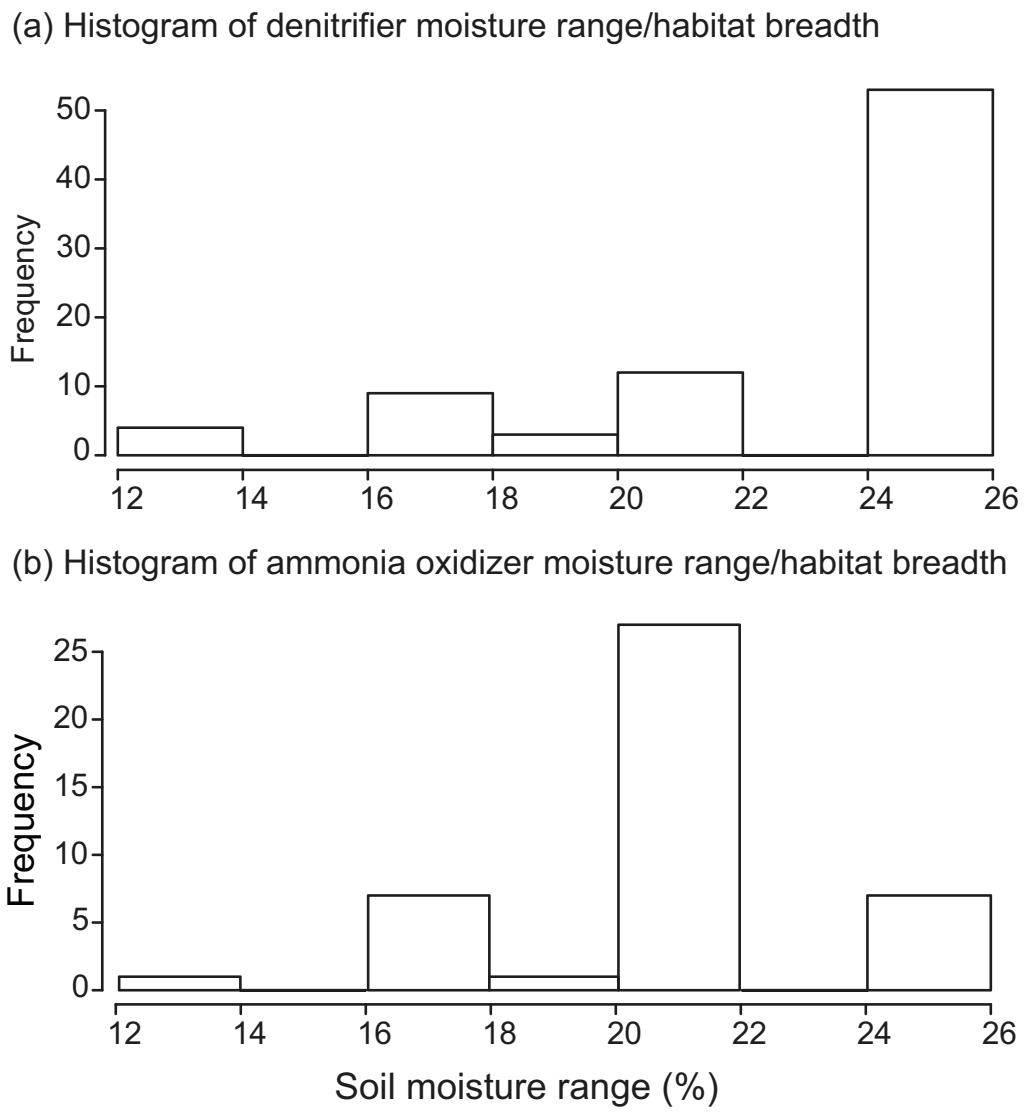


Figure B.3. Histogram of denitrifier (a) and ammonia oxidizers (b) moisture range/habitat breadth (maximum-minimum) along the moisture gradient. A taxon was considered present if detected in two out of four replicate plots along the gradient.

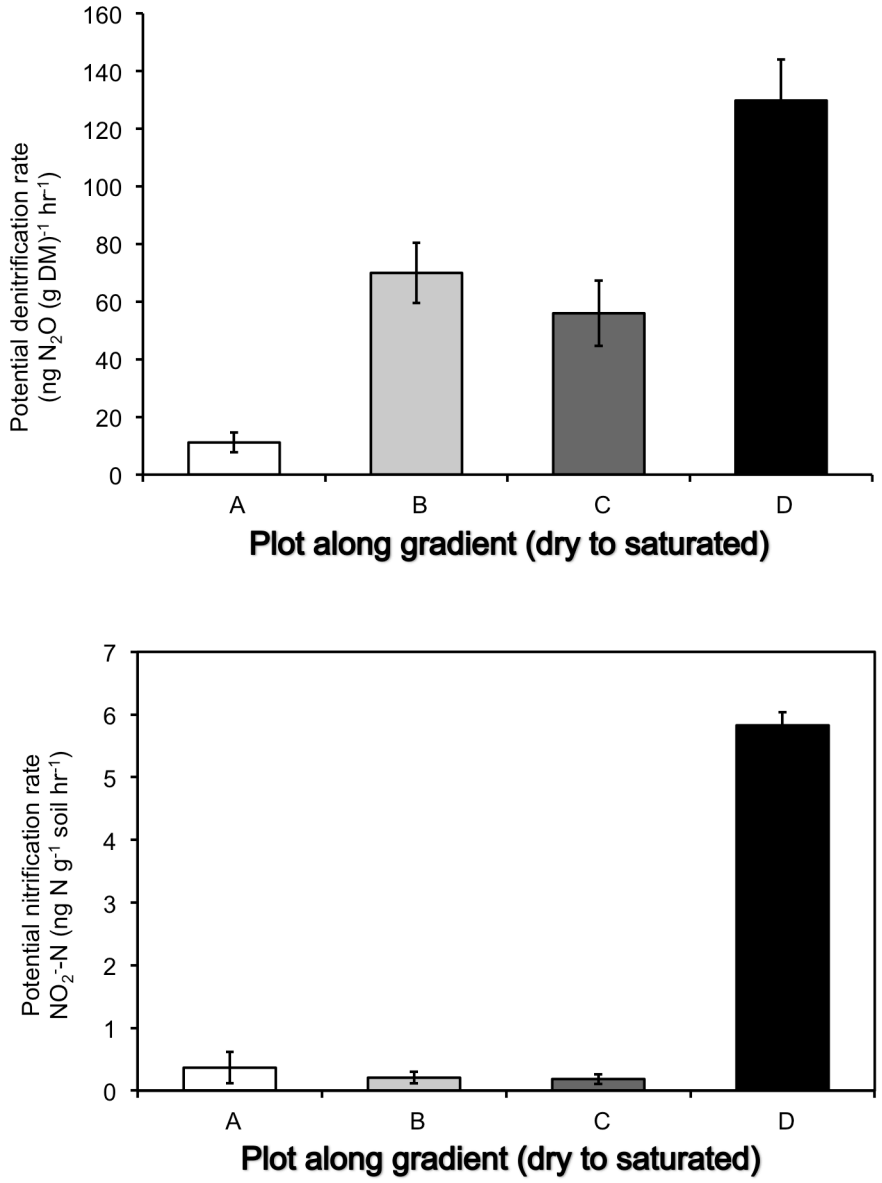


Figure B.4. Potential denitrification (top) and nitrification (bottom) rates (mean +/- standard error) along the moisture gradient for soils collected during the September sampling. Bars are colored according to location along the gradient from dry (white bar) to saturated (black bar) plots (labeled plots A through D).

APPENDIX C

SUPPLEMENTARY INFORMATION FOR CHAPTER 4

Table C.1. Summary of permutational MANOVA (PERMANOVA) results. Contribution of plot along upland to wetland gradient on plant community variation at wetland sites IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d) is presented. Effects were considered significant to contributing to community variation at $P < 0.05$. (Abbrevations: Df = degrees of freedom, Sums Sqs = sums of squares, Mean Sqs = mean squares, F.Model = *F* statistic)

(a) IL-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	2.7454	0.9152	7.8491	0.6624	0.0010
Residuals	12	1.3991	0.1166	0.3376		
Total	15	4.1445	1.0000			

(b) IL-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	2.7471	0.9157	7.8976	0.6638	0.0010
Residuals	12	1.3914	0.1160	0.3362		
Total	15	4.1385	1.0000			

(c) MI-1

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	3.7867	1.2622	9.4287	0.7021	0.0010
Residuals	12	1.6065	0.1339	0.2979		
Total	15	5.3932	1.0000			

(d) MI-2

Effect	Df	Sums Sqs	Mean Sqs	F.Model	R^2	*P*-value
Plot	3	3.4226	1.1409	5.8052	0.5921	0.0010
Residuals	12	2.3583	0.1965	0.4080		
Total	15	5.7809	1.0000			

Table C.2. Summary of soil factors (mean, standard deviation = stdev, minimum =min, and maximum = max) along the upland to wetland gradient (plots A – D) at IL-1 (a), IL-2 (b), MI-1 (c) and MI-2 (d). Soil texture (%sand, %silt, %clay), pH and soil organic matter (total organic C = TOC, total nitrogen = TN, and C:N ratio = CN) were averaged along the gradient over all sampling months.

(a) IL-1

	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
sand	50	10	41	66	45	11	28	56	31	5	23	36	21	6	12	26
silt	29	6	19	35	33	8	26	44	42	4	38	49	47	4	42	53
clay	21	4	15	24	22	4	18	28	27	1	26	28	33	2	31	35
pH	7.68	0.79	5.55	8.37	8.14	0.45	7.09	8.68	8.10	0.34	7.32	8.39	8.05	0.31	7.40	8.37
TOC	14.59	2.49	10.50	18.00	19.18	1.99	16.80	23.70	27.07	1.99	23.10	30.60	32.38	2.86	29.10	39.10
TN	1.16	0.20	0.90	1.40	1.42	0.21	1.10	1.80	1.86	0.16	1.60	2.10	2.22	0.37	1.80	3.20
CN	12.73	2.21	10.50	17.20	13.67	1.18	11.60	15.50	14.60	0.56	13.60	15.60	14.77	1.40	12.20	17.00

(b) IL-2

	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
sand	26	6	18	34	33	5	27	39	33	3	28	37	45	8	36	58
silt	50	4	44	54	42	4	38	47	43	2	41	45	35	5	27	41
clay	25	2	22	28	25	1	23	26	25	2	22	27	20	3	15	23
pH	5.72	0.31	5.16	6.24	7.56	0.22	7.03	7.86	7.38	0.49	6.73	7.99	7.17	0.18	6.81	7.42
TOC	14.13	1.10	12.3	16.1	14.84	3.08	11.8	21.8	14.24	3.14	10	19.1	7.17	1.38	5	9.3
TN	1.27	0.09	1.1	1.4	1.15	0.07	1.1	1.3	1.30	0.21	1	1.6	0.73	0.12	0.5	0.9
CN	11.17	0.64	9.7	12.4	12.88	2.30	10.2	18.2	10.86	0.82	9.8	12.1	9.87	0.69	9	11

Table C.2 (cont.)

(c) MI-1

	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
sand	74	3	70	78	87	3	82	89	87	3	83	90	86	3	83	90
silt	16	2	13	17	8	2	6	11	8	2	5	11	9	2	5	10
clay	10	2	9	13	5	1	4	7	6	0	5	6	6	1	5	7
pH	5.21	0.32	4.65	5.71	7.32	0.18	6.92	7.45	7.16	0.29	6.54	7.52	6.84	0.19	6.47	7.05
TOC	27.58	9.55	13.60	46.20	15.90	6.78	9.80	31.80	6.28	1.49	4.20	9.10	8.28	2.80	4.30	13.80
TN	1.43	0.43	0.80	2.20	1.19	0.39	0.70	1.80	0.45	0.10	0.30	0.60	0.54	0.16	0.30	0.80
CN	19.05	1.52	16.30	21.00	13.32	2.52	12.30	18.00	14.00	1.46	12.20	16.40	15.18	1.19	13.40	17.30

(d) MI-2

	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
sand	59	10	43	70	60	3	56	62	66	4	60	71	60	20	34	85
silt	28	9	18	41	30	2	28	33	27	4	22	32	31	17	10	53
clay	14	2	11	16	10	1	9	11	8	1	7	9	8	3	5	13
pH	6.23	0.14	5.93	6.46	6.05	0.27	5.51	6.42	6.28	0.17	5.95	6.51	6.47	0.14	6.23	6.67
TOC	21.65	2.04	17.30	24.30	19.99	2.06	16.90	23.00	22.44	5.95	15.90	34.60	23.44	8.04	8.40	35.80
TN	1.69	0.17	1.40	2.10	1.59	0.20	1.30	2.00	1.63	0.32	1.20	2.20	1.69	0.68	0.60	2.70
CN	12.83	0.90	11.60	15.10	12.62	0.83	11.50	14.10	13.69	1.12	11.60	15.70	14.22	1.60	12.70	18.20

(UNITS: sand, silt, clay: %; TOC: g C kg^{-1} soil; TN: g N kg^{-1} soil; CN: wt/wt; moisture: %; ammonium: µg NH_4^+-N g^{-1} soil; nitrate: µg NO_3^--N g^{-1} soil)

Table C.3. Summary of soil factors (mean, standard deviation = stdev, minimum = min, and maximum = max) along the upland to wetland gradient (plots A – D) at wetland IL-1 (a), IL-2 (b), MI-1 (c) and MI-2 (d). Soil moisture and inorganic N (ammonium, nitrate) were averaged along the upland to wetland gradient for each sampling month.

(a) IL-1

June	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	17.84	4.08	14.25	23.58	26.75	2.65	23.71	30.09	42.06	2.75	40.08	46.01	44.48	6.08	39.47	53.34
ammonium	0.838	0.025	0.016	0.017	1.088	0.325	0.016	0.031	2.425	0.807	0.025	0.06	2.700	0.634	0.036	0.065
nitrate	1.650	0.311	0.028	0.042	2.000	0.204	0.036	0.044	3.025	0.301	0.052	0.066	2.500	0.381	0.043	0.058
July	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	17.00	2.46	14.12	19.84	24.08	3.19	21.36	28.69	37.25	5.90	29.92	44.02	40.17	7.44	31.52	47.47
ammonium	0.350	0.108	0.004	0.009	0.325	0.087	0.005	0.009	0.475	0.206	0.005	0.013	0.438	0.197	0.003	0.012
nitrate	3.963	0.634	0.062	0.091	3.550	0.917	0.052	0.09	5.675	1.796	0.079	0.164	4.975	1.019	0.07	0.116
August	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	10.89	2.03	8.36	12.58	19.44	4.61	13.81	24.41	34.28	4.27	29.36	39.2	35.98	2.83	32.8	39.47
ammonium	0.500	0.183	0.006	0.014	0.400	0.147	0.005	0.011	0.575	0.132	0.009	0.015	0.788	0.284	0.012	0.024
nitrate	1.925	0.272	0.034	0.046	2.200	0.358	0.037	0.054	3.350	0.402	0.057	0.074	4.238	0.686	0.069	0.102

Table C.3 (cont.)

(b) IL-2

June	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	23.95	2.68	21.89	27.88	24.63	2.12	22.24	27.12	30.81	4.03	26.1	35.93	30.02	3.60	26.85	34.62
ammonium	3.675	3.960	0.026	0.192	3.138	0.687	0.046	0.074	5.788	0.757	0.102	0.137	10.313	4.920	0.148	0.353
nitrate	1.338	0.609	0.02	0.045	2.025	1.317	0.027	0.08	1.438	0.184	0.024	0.033	1.300	0.082	0.024	0.028
July	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	30.58	6.79	25.97	40.68	26.10	3.14	21.62	28.28	33.61	4.70	29.88	40.44	30.44	2.26	28.11	33.48
ammonium	1.575	0.444	0.02	0.041	2.963	1.998	0.031	0.117	6.950	3.992	0.069	0.251	17.438	6.427	0.209	0.49
nitrate	3.300	1.340	0.044	0.105	2.988	0.899	0.045	0.085	2.663	1.430	0.024	0.087	2.763	1.274	0.032	0.082
August	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	20.21	3.73	15.54	24.66	24.01	2.62	20.2	26.03	30.92	2.45	28.73	33.51	26.01	0.97	24.91	27.15
ammonium	0.700	0.261	0.007	0.018	3.588	1.920	0.04	0.126	4.625	1.963	0.058	0.147	11.763	2.464	0.192	0.286
nitrate	2.113	0.206	0.037	0.047	2.238	0.206	0.041	0.05	2.175	0.545	0.03	0.055	1.850	0.238	0.032	0.042

Table C.3 (cont.)

(c) MI-1

June	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	7.03	1.97	4.73	9.42	N/A				34.97	20.43	23.96	65.57	32.45	16.51	20.5	56.76
ammonium	0.988	0.225	0.014	0.025					1.375	0.797	0.01	0.042	0.513	0.278	0.004	0.017
nitrate	1.100	0.235	0.016	0.027					1.675	0.753	0.025	0.056	1.663	0.757	0.022	0.055
July	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	7.67	1.56	6.09	9.61	40.83	7.62	32.9	48.3	23.54	3.07	20.43	27.29	37.26	16.36	26.51	61.4
ammonium	1.288	0.578	0.012	0.038	10.763	4.098	0.128	0.325	1.000	0.520	0.009	0.033	0.763	0.379	0.009	0.026
nitrate	1.263	0.125	0.024	0.029	2.838	1.055	0.037	0.085	1.400	0.242	0.024	0.035	1.913	0.867	0.024	0.063
August	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	11.99	1.11	10.91	13.45	33.84	4.25	30.36	39.32	25.63	2.61	22.22	28.58	26.36	0.89	25.52	27.43
ammonium	0.613	0.189	0.008	0.017	4.838	1.063	0.071	0.123	1.388	0.814	0.013	0.051	0.713	0.612	0.004	0.032
nitrate	2.725	0.939	0.03	0.072	4.300	0.673	0.07	0.102	2.213	0.229	0.039	0.05	2.100	0.363	0.034	0.051

Table C.3 (cont.)

(d) MI-2

June	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	11.33	3.09	7.91	15.26	13.45	2.04	10.87	15.11	58.97	16.23	42.04	81.10	53.83	23.24	27.26	73.96
ammonium	1.338	0.986	0.013	0.056	1.700	0.587	0.018	0.045	10.175	5.444	0.078	0.339	2.725	2.306	0.010	0.119
nitrate	0.988	0.118	0.018	0.023	1.063	0.206	0.016	0.026	2.475	1.577	0.023	0.095	1.638	0.871	0.018	0.054
July	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	7.95	2.29	6.14	11.10	9.54	1.95	7.14	11.21	38.22	4.37	32.59	43.16	52.89	5.98	47.94	59.54
ammonium	0.813	0.547	0.007	0.031	1.038	0.295	0.015	0.028	3.563	1.087	0.044	0.096	3.367	2.181	0.017	0.094
nitrate	1.613	0.217	0.028	0.038	1.463	0.225	0.023	0.033	2.450	0.443	0.038	0.058	2.367	0.416	0.038	0.054
August	A				B				C				D			
soil factor	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max	mean	stdev	min	max
moisture	18.34	4.18	13.47	22.77	22.38	2.78	18.81	25.60	38.00	7.92	29.74	48.79	45.31	11.83	27.58	51.87
ammonium	1.113	0.933	0.010	0.050	4.725	6.357	0.024	0.285	7.150	5.794	0.057	0.314	1.875	1.234	0.006	0.060
nitrate	1.300	0.220	0.021	0.031	1.675	0.307	0.026	0.041	1.713	0.415	0.023	0.043	1.913	0.304	0.033	0.047

Table C.4. *Post-hoc* contrasts comparing all combinations of plot*month. All comparisons of potential denitrification and nitrification rates are reported according to site: IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d). Comparisons were considered signficant at $P < 0.05$.

(a) IL-1 - denitrification

plot	month	plot	month	Adj *P*
A	6	B	6	0.8776
A	6	C	6	0.0109
A	6	D	6	0.0196
A	6	A	7	<0.0001
A	6	B	7	<0.0001
A	6	C	7	<0.0001
A	6	D	7	<0.0001
A	6	A	8	<0.0001
A	6	B	8	<0.0001
A	6	C	8	0.0009
A	6	D	8	0.0033
B	6	C	6	0.3927
B	6	D	6	0.5335
B	6	A	7	<0.0001
B	6	B	7	<0.0001
B	6	C	7	<0.0001
B	6	D	7	<0.0001
B	6	A	8	<0.0001
B	6	B	8	<0.0001
B	6	C	8	<0.0001
B	6	D	8	<0.0001
C	6	D	6	1.0000
C	6	A	7	<0.0001
C	6	B	7	<0.0001
C	6	C	7	<0.0001
C	6	D	7	<0.0001
C	6	A	8	<0.0001
C	6	B	8	<0.0001
C	6	C	8	<0.0001
C	6	D	8	<0.0001

(a) IL-1 - nitrification

plot	month	plot	month	Adj *P*
A	6	B	6	0.6061
A	6	C	6	0.0014
A	6	D	6	<0.0001
A	6	A	7	1.0000
A	6	B	7	0.9170
A	6	C	7	0.0813
A	6	D	7	0.0065
A	6	A	8	1.0000
A	6	B	8	0.8089
A	6	C	8	0.0037
A	6	D	8	0.0008
B	6	C	6	0.2542
B	6	D	6	0.0070
B	6	A	7	0.6725
B	6	B	7	0.9973
B	6	C	7	0.9880
B	6	D	7	0.5731
B	6	A	8	0.6791
B	6	B	8	1.0000
B	6	C	8	0.4432
B	6	D	8	0.1749
C	6	D	6	0.9149
C	6	A	7	0.0019
C	6	B	7	0.0772
C	6	C	7	0.3229
C	6	D	7	1.0000
C	6	A	8	0.0019
C	6	B	8	0.1334
C	6	C	8	1.0000
C	6	D	8	1.0000

Table C.4 (cont.)

(a) IL-1 - denitrification

plot	month	plot	month	Adj *P*
D	6	A	7	<0.0001
D	6	B	7	<0.0001
D	6	C	7	<0.0001
D	6	D	7	<0.0001
D	6	A	8	<0.0001
D	6	B	8	<0.0001
D	6	C	8	<0.0001
D	6	D	8	<0.0001
A	7	B	7	1.0000
A	7	C	7	0.9994
A	7	D	7	0.3029
A	7	A	8	0.0241
A	7	B	8	0.9246
A	7	C	8	0.8280
A	7	D	8	0.5516
B	7	C	7	0.9998
B	7	D	7	0.3445
B	7	A	8	0.0312
B	7	B	8	0.9439
B	7	C	8	0.7866
B	7	D	8	0.5003
C	7	D	7	0.8015
C	7	A	8	0.1656
C	7	B	8	0.9999
C	7	C	8	0.3206
C	7	D	8	0.1426
D	7	A	8	0.9892
D	7	B	8	0.9918
D	7	C	8	0.0053
D	7	D	8	0.0014
A	8	B	8	0.5173
A	8	C	8	0.0002
A	8	D	8	<0.0001
B	8	C	8	0.0855
B	8	D	8	0.0292
C	8	D	8	1.0000

(a) IL-1 - nitrification

plot	month	plot	month	Adj *P*
D	6	A	7	<0.0001
D	6	B	7	0.0014
D	6	C	7	0.1215
D	6	D	7	0.0534
D	6	A	8	<0.0001
D	6	B	8	0.0028
D	6	C	8	0.7513
D	6	D	8	0.5383
A	7	B	7	0.9462
A	7	C	7	0.1018
A	7	D	7	0.0086
A	7	A	8	1.0000
A	7	B	8	0.8589
A	7	C	8	0.0050
A	7	D	8	0.0011
B	7	C	7	0.8284
B	7	D	7	0.2387
B	7	A	8	0.9487
B	7	B	8	1.0000
B	7	C	8	0.1625
B	7	D	8	0.0486
C	7	D	7	0.9963
C	7	A	8	0.1042
C	7	B	8	0.9289
C	7	C	8	0.6820
C	7	D	8	0.8242
D	7	A	8	0.0088
D	7	B	8	0.3649
D	7	C	8	1.0000
D	7	D	8	0.9801
A	8	B	8	0.8635
A	8	C	8	0.0051
A	8	D	8	0.0011
B	8	C	8	0.2611
B	8	D	8	0.0868
C	8	D	8	1.0000

Table C.4 (cont.)

(b) IL-2 - denitrification

plot	month	plot	month	Adj *P*
A	6	B	6	0.9798
A	6	C	6	0.9871
A	6	D	6	0.552
A	6	A	7	0.3998
A	6	B	7	0.5246
A	6	C	7	1.0000
A	6	D	7	1.0000
A	6	A	8	0.1599
A	6	B	8	0.0946
A	6	C	8	0.0904
A	6	D	8	0.0680
B	6	C	6	1.0000
B	6	D	6	0.0557
B	6	A	7	0.0143
B	6	B	7	0.1205
B	6	C	7	0.9987
B	6	D	7	0.7722
B	6	A	8	0.0029
B	6	B	8	0.0154
B	6	C	8	0.0039
B	6	D	8	0.0027
C	6	D	6	0.0658
C	6	A	7	0.0173
C	6	B	7	0.0597
C	6	C	7	0.9998
C	6	D	7	0.8114
C	6	A	8	0.0036
C	6	B	8	0.0050
C	6	C	8	0.0173
C	6	D	8	0.0033

(b) IL-2 - nitrification

plot	month	plot	month	Adj *P*
A	6	B	6	<0.0001
A	6	C	6	0.0006
A	6	D	6	0.9987
A	6	A	7	1.0000
A	6	B	7	<0.0001
A	6	C	7	0.0578
A	6	D	7	0.9729
A	6	A	8	1.0000
A	6	B	8	0.0001
A	6	C	8	0.3434
A	6	D	8	0.9988
B	6	C	6	0.8579
B	6	D	6	<0.0001
B	6	A	7	<0.0001
B	6	B	7	1.0000
B	6	C	7	0.0631
B	6	D	7	0.0002
B	6	A	8	<0.0001
B	6	B	8	0.8485
B	6	C	8	0.0066
B	6	D	8	<0.0001
C	6	D	6	0.0074
C	6	A	7	0.0004
C	6	B	7	0.8495
C	6	C	7	0.2890
C	6	D	7	0.0211
C	6	A	8	0.0004
C	6	B	8	1.0000
C	6	C	8	0.0132
C	6	D	8	0.0071

Table C.4 (cont.)

(b) IL-2 - denitrification

plot	month	plot	month	Adj *P*
D	6	A	7	1.0000
D	6	B	7	1.0000
D	6	C	7	0.3374
D	6	D	7	0.9499
D	6	A	8	0.9902
D	6	B	8	0.9961
D	6	C	8	0.9954
D	6	D	8	0.9955
A	7	B	7	1.0000
A	7	C	7	0.1213
A	7	D	7	0.5931
A	7	A	8	1.0000
A	7	B	8	1.0000
A	7	C	8	1.0000
A	7	D	8	1.0000
B	7	C	7	0.3152
B	7	D	7	0.8797
B	7	A	8	0.9928
B	7	B	8	0.9990
B	7	C	8	0.9968
B	7	D	8	0.9912
C	7	D	7	0.9975
C	7	A	8	0.0313
C	7	B	8	0.0420
C	7	C	8	0.1002
C	7	D	8	0.0293
D	7	A	8	0.2514
D	7	B	8	0.3095
D	7	C	8	0.2990
D	7	D	8	0.3970
A	8	B	8	1.0000
A	8	C	8	1.0000
A	8	D	8	1.0000
B	8	C	8	1.0000
B	8	D	8	1.0000
C	8	D	8	1.0000

(b) IL-2 - nitrification

plot	month	plot	month	Adj *P*
D	6	A	7	0.9955
D	6	B	7	<0.0001
D	6	C	7	0.3472
D	6	D	7	1.0000
D	6	A	8	0.9944
D	6	B	8	0.0015
D	6	C	8	0.8753
D	6	D	8	1.0000
A	7	B	7	<0.0001
A	7	C	7	0.0422
A	7	D	7	0.9469
A	7	A	8	1.0000
A	7	B	8	<0.0001
A	7	C	8	0.2752
A	7	D	8	0.9961
B	7	C	7	0.0605
B	7	D	7	0.0002
B	7	A	8	<0.0001
B	7	B	8	0.8349
B	7	C	8	0.0063
B	7	D	8	<0.0001
C	7	D	7	0.5964
C	7	A	8	0.0394
C	7	B	8	0.4932
C	7	C	8	0.9585
C	7	D	8	0.3390
D	7	A	8	0.9398
D	7	B	8	0.0045
D	7	C	8	0.9797
D	7	D	8	0.9999
A	8	B	8	<0.0001
A	8	C	8	0.2619
A	8	D	8	0.9950
B	8	C	8	0.1017
B	8	D	8	0.0014
C	8	D	8	0.8689

Table C.4 (cont.)

(c) MI-1 - denitrification

plot	month	plot	month	Adj *P*
A	6	C	6	0.9921
A	6	D	6	0.9995
A	6	A	7	0.1031
A	6	B	7	0.9992
A	6	C	7	0.0771
A	6	D	7	0.3253
A	6	A	8	0.0122
A	6	B	8	0.7509
A	6	C	8	0.0077
A	6	D	8	0.0431
C	6	D	6	1.0000
C	6	A	7	0.0033
C	6	B	7	0.7568
C	6	C	7	0.0198
C	6	D	7	0.0399
C	6	A	8	0.0002
C	6	B	8	0.1791
C	6	C	8	0.0022
C	6	D	8	0.0031
D	6	A	7	0.0070
D	6	B	7	0.8965
D	6	C	7	0.0130
D	6	D	7	0.1600
D	6	A	8	0.0005
D	6	B	8	0.3006
D	6	C	8	0.0011
D	6	D	8	0.0214
A	7	B	7	0.2304
A	7	C	7	1.0000
A	7	D	7	0.9946
A	7	A	8	0.9972
A	7	B	8	0.8295
A	7	C	8	0.9996
A	7	D	8	1.0000

(c) MI-1 - nitrification

plot	month	plot	month	Adj *P*
A	6	C	6	0.9996
A	6	D	6	1.0000
A	6	A	7	0.5481
A	6	B	7	<0.0001
A	6	C	7	1.0000
A	6	D	7	1.0000
A	6	A	8	1.0000
A	6	B	8	<0.0001
A	6	C	8	1.0000
A	6	D	8	1.0000
C	6	D	6	1.0000
C	6	A	7	1.0000
C	6	B	7	<0.0001
C	6	C	7	0.9939
C	6	D	7	1.0000
C	6	A	8	0.9999
C	6	B	8	<0.0001
C	6	C	8	0.9676
C	6	D	8	1.0000
D	6	A	7	0.9984
D	6	B	7	<0.0001
D	6	C	7	1.0000
D	6	D	7	1.0000
D	6	A	8	1.0000
D	6	B	8	<0.0001
D	6	C	8	1.0000
D	6	D	8	1.0000
A	7	B	7	<0.0001
A	7	C	7	0.9870
A	7	D	7	0.9989
A	7	A	8	0.6764
A	7	B	8	<0.0001
A	7	C	8	0.9682
A	7	D	8	0.9958

Table C.4 (cont.)

(c) MI-1 - denitrification

plot	month	plot	month	Adj *P*
B	7	C	7	0.3442
B	7	D	7	0.7991
B	7	A	8	0.0259
B	7	B	8	0.9964
B	7	C	8	0.0531
B	7	D	8	0.2235
C	7	D	7	0.9994
C	7	A	8	0.9693
C	7	B	8	0.9250
C	7	C	8	0.9984
C	7	D	8	1.0000
D	7	A	8	0.6459
D	7	B	8	0.9997
D	7	C	8	0.8228
D	7	D	8	0.9975
A	8	B	8	0.2440
A	8	C	8	1.0000
A	8	D	8	0.9941
B	8	C	8	0.3984
B	8	D	8	0.8211
C	8	D	8	0.9997

(c) MI-1 - nitrification

plot	month	plot	month	Adj *P*
B	7	C	7	<0.0001
B	7	D	7	<0.0001
B	7	A	8	<0.0001
B	7	B	8	1.0000
B	7	C	8	<0.0001
B	7	D	8	<0.0001
C	7	D	7	1.0000
C	7	A	8	1.0000
C	7	B	8	<0.0001
C	7	C	8	1.0000
C	7	D	8	1.0000
D	7	A	8	1.0000
D	7	B	8	<0.0001
D	7	C	8	1.0000
D	7	D	8	1.0000
A	8	B	8	<0.0001
A	8	C	8	1.0000
A	8	D	8	1.0000
B	8	C	8	<0.0001
B	8	D	8	<0.0001
C	8	D	8	1.0000

Table C.4 (cont.)

(d) MI-2 - denitrification

plot	month	plot	month	Adj *P*
A	6	B	6	0.9937
A	6	C	6	0.4120
A	6	D	6	0.8679
A	6	A	7	<0.0001
A	6	B	7	0.0633
A	6	C	7	0.9999
A	6	D	7	0.4233
A	6	A	8	<0.0001
A	6	B	8	0.0057
A	6	C	8	0.5956
A	6	D	8	0.0690
B	6	C	6	0.9648
B	6	D	6	1.0000
B	6	A	7	0.0003
B	6	B	7	<0.0001
B	6	C	7	0.8334
B	6	D	7	0.0576
B	6	A	8	<0.0001
B	6	B	8	<0.0001
B	6	C	8	0.0974
B	6	D	8	0.0046
C	6	D	6	0.9997
C	6	A	7	<0.0001
C	6	B	7	<0.0001
C	6	C	7	0.0002
C	6	D	7	0.0019
C	6	A	8	<0.0001
C	6	B	8	<0.0001
C	6	C	8	<0.0001
C	6	D	8	0.0001

(d) MI-2 - nitrification

plot	month	plot	month	Adj *P*
A	6	B	6	0.5510
A	6	C	6	0.9999
A	6	D	6	1.0000
A	6	A	7	0.8940
A	6	B	7	0.9996
A	6	C	7	1.0000
A	6	D	7	1.0000
A	6	A	8	1.0000
A	6	B	8	0.9011
A	6	C	8	1.0000
A	6	D	8	1.0000
B	6	C	6	0.9201
B	6	D	6	0.8751
B	6	A	7	0.9987
B	6	B	7	0.8434
B	6	C	7	0.8606
B	6	D	7	0.4447
B	6	A	8	0.6458
B	6	B	8	0.9997
B	6	C	8	0.8510
B	6	D	8	0.5830
C	6	D	6	1.0000
C	6	A	7	0.9999
C	6	B	7	1.0000
C	6	C	7	1.0000
C	6	D	7	0.9979
C	6	A	8	1.0000
C	6	B	8	0.9986
C	6	C	8	1.0000
C	6	D	8	1.0000

Table C.4 (cont.)

(d) MI-2 - denitrification

plot	month	plot	month	Adj *P*
D	6	A	7	<0.0001
D	6	B	7	0.0008
D	6	C	7	0.4703
D	6	D	7	<0.0001
D	6	A	8	<0.0001
D	6	B	8	<0.0001
D	6	C	8	0.0242
D	6	D	8	<0.0001
A	7	B	7	0.9980
A	7	C	7	0.0335
A	7	D	7	0.8103
A	7	A	8	0.6753
A	7	B	8	1.0000
A	7	C	8	0.5546
A	7	D	8	0.9972
B	7	C	7	0.2500
B	7	D	7	0.9989
B	7	A	8	0.6565
B	7	B	8	0.8321
B	7	C	8	0.9774
B	7	D	8	1.0000
C	7	D	7	0.8216
C	7	A	8	0.0018
C	7	B	8	0.0318
C	7	C	8	0.2777
C	7	D	8	0.2670
D	7	A	8	0.2005
D	7	B	8	0.7995
D	7	C	8	1.0000
D	7	D	8	0.9471
A	8	B	8	0.9930
A	8	C	8	0.0783
A	8	D	8	0.6323
B	8	C	8	0.5407
B	8	D	8	0.9966
C	8	D	8	0.9819

(d) MI-2 - nitrification

plot	month	plot	month	Adj *P*
D	6	A	7	0.9997
D	6	B	7	1.0000
D	6	C	7	1.0000
D	6	D	7	0.9970
D	6	A	8	1.0000
D	6	B	8	0.9957
D	6	C	8	1.0000
D	6	D	8	1.0000
A	7	B	7	1.0000
A	7	C	7	0.9996
A	7	D	7	0.9152
A	7	A	8	0.9484
A	7	B	8	1.0000
A	7	C	8	0.9994
A	7	D	8	0.9768
B	7	C	7	1.0000
B	7	D	7	0.9948
B	7	A	8	0.9999
B	7	B	8	0.9975
B	7	C	8	1.0000
B	7	D	8	0.9998
C	7	D	7	0.9995
C	7	A	8	1.0000
C	7	B	8	0.9944
C	7	C	8	1.0000
C	7	D	8	1.0000
D	7	A	8	1.0000
D	7	B	8	0.8089
D	7	C	8	0.9996
D	7	D	8	1.0000
A	8	B	8	0.9454
A	8	C	8	1.0000
A	8	D	8	1.0000
B	8	C	8	0.9935
B	8	D	8	0.9179
C	8	D	8	1.0000

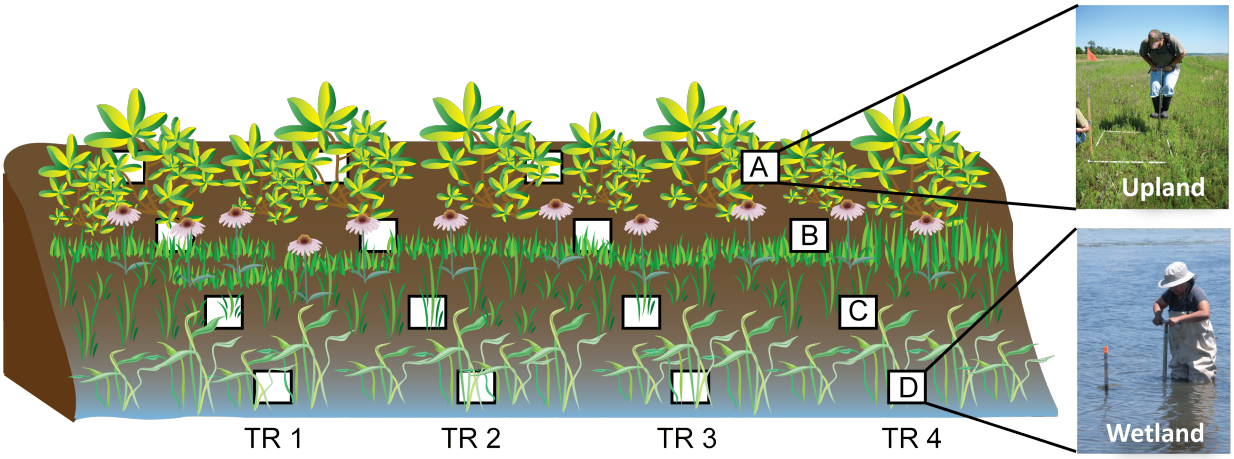


Figure C.1. Experimental design of soil sampling at each wetland site. Soil samples were collected monthly from June-August 2009 over four transects (TR), each composed of four plots along an upland to wetland gradient (plots A-D). .

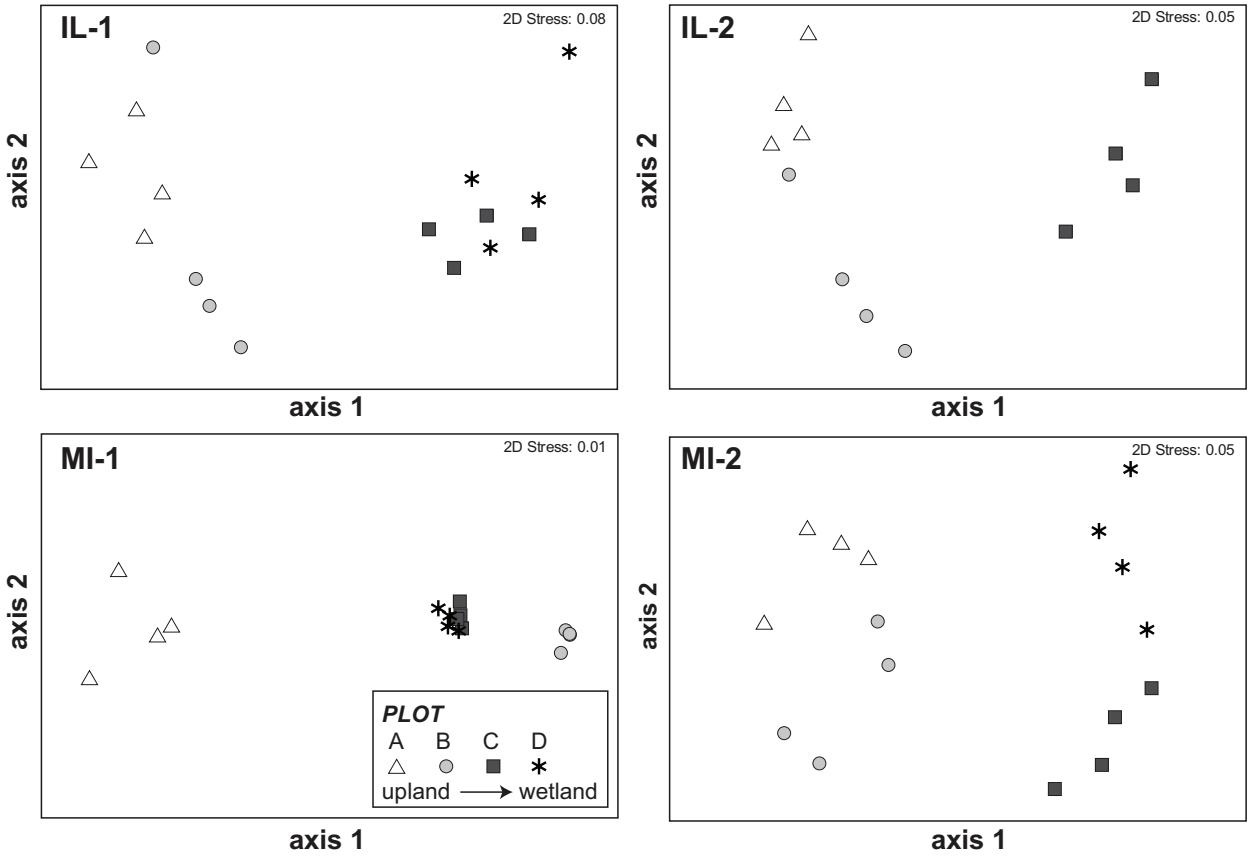


Figure C.2. Non-metric multidimensional scaling analysis for plant community composition at IL-1 (a), IL-2 (b), MI-1 (c), and MI-2 (d)) within 3 1-m^2 plots for Illinois wetlands and 1-2 1-m^2 plots for Michigan wetlands at each transect along an upland to wetland gradient. Plots with similar plant community composition plot closer together. Sample color (white to black) corresponds to location of sample along the gradient. At IL-2, no plants were observed in the most saturated plot D.

Details of statistics used to analyze plant community composition at each wetland site.

Permutational multivariate analysis of variance (PERMANOVA) using the adonis function was used to assess the influence of location along the gradient on plant community composition within each wetland site. PERMANOVA was carried out using the R Statistics Package (R Development Core Team 2011). Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship among plant communities along the moisture gradient using PRIMER version 6 (PRIMER-E Ltd, Plymouth, UK).

APPENDIX D

SUPPLEMENTARY INFORMATION FOR CHAPTER 5

Table D.1. *Post-hoc* contrasts comparing all combinations of history*drying/flooding treatment. All history*treatment comparisons of potential denitrification (a) and nitrification (b) rates are reported. Comparisons were considered signficant at $P < 0.05$.

(a) Denitrification

history	treatment		history	treatment	adj. *P*-value
Upland	Dry	vs.	Upland	Wet-Dry	0.0167
Upland	Dry	vs.	Upland	Wet	<0.0001
Upland	Dry	vs.	Wetland	Dry	0.2334
Upland	Dry	vs.	Wetland	Wet-Dry	<0.0001
Upland	Dry	vs.	Wetland	Wet	<0.0001
Upland	Wet-Dry	vs.	Upland	Wet	0.1277
Upland	Wet-Dry	vs.	Wetland	Dry	0.0001
Upland	Wet-Dry	vs.	Wetland	Wet-Dry	<0.0001
Upland	Wet-Dry	vs.	Wetland	Wet	0.0011
Upland	Wet	vs.	Wetland	Dry	<0.0001
Upland	Wet	vs.	Wetland	Wet-Dry	0.0074
Upland	Wet	vs.	Wetland	Wet	0.2396
Wetland	Dry	vs.	Wetland	Wet-Dry	<0.0001
Wetland	Dry	vs.	Wetland	Wet	<0.0001
Wetland	Wet-Dry	vs.	Wetland	Wet	0.4929

(b) Nitrification

history	treatment		history	treatment	adj. *P*-value
Upland	Dry	vs.	Upland	Wet-Dry	0.9991
Upland	Dry	vs.	Upland	Wet	0.3912
Upland	Dry	vs.	Wetland	Dry	0.0010
Upland	Dry	vs.	Wetland	Wet-Dry	0.9953
Upland	Dry	vs.	Wetland	Wet	0.9998
Upland	Wet-Dry	vs.	Upland	Wet	0.5964
Upland	Wet-Dry	vs.	Wetland	Dry	0.0021
Upland	Wet-Dry	vs.	Wetland	Wet-Dry	1.0000
Upland	Wet-Dry	vs.	Wetland	Wet	1.0000
Upland	Wet	vs.	Wetland	Dry	0.0601
Upland	Wet	vs.	Wetland	Wet-Dry	0.6853
Upland	Wet	vs.	Wetland	Wet	0.5324
Wetland	Dry	vs.	Wetland	Wet-Dry	0.0028
Wetland	Dry	vs.	Wetland	Wet	0.0017
Wetland	Wet-Dry	vs.	Wetland	Wet	0.9998

Table D.2. Summary of qPCR results (log copies gene ng^{-1} DNA) (mean ± SD) for baseline and post-drying/flooding treatments.

History	Treatment	NOSqPCR	AOAqPCR	AOBqPCR
Upland	Baseline	4.785 ± 0.146	12.417 ± 0.130	4.705 ± 0.089
	Dry	5.054 ± 0.048	12.814 ± 0.065	4.842 ± 0.207
	W/D	4.986 ± 0.114	12.753 ± 0.105	4.727 ± 0.088
	Wet	5.033 ± 0.206	12.957 ± 0.433	4.863 ± 0.202
Wetland	Baseline	4.982 ± 0.172	13.149 ± 0.320	4.475 ± 0.170
	Dry	5.031 ± 0.090	12.808 ± 0.072	4.488 ± 0.072
	W/D	5.022 ± 0.048	12.892 ± 0.198	4.404 ± 0.085
	Wet	4.938 ± 0.129	12.751 ± 0.120	4.329 ± 0.104